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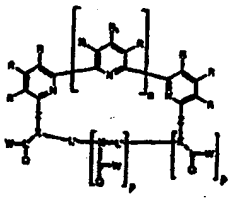

(I)

(57) Abstract

A targeting immunoreagent comprising a metal ion, a residue of a complexing agent and an immunoreactive group linked to said complNxing agent having structure (I), wherein each R and R_1 is independently selected from hydrogen, alkyl, alkoxy, hydroxyalkyl, alkoxyalkyl, hydroxyalkyloxy, alkoxyalkyloxy, alkylthio, alkylthioalkyl, alkylthioalkyloxy, hydroxyalkylthio, hydroxyalkylthioalkyl, hydroxyalkylthioalkyloxy, N,N—dialkylamino, N-(hydroxyalkyl)-N-alkylamino, N,N—bis(hydroyalkyl)amino, N,N-dialkylaminoalkyl, N—(hydroxyalkyl)-N-alkylaminoalkyl, N-N-bis(hydroxyalkyloaminoalkyl), alkylformamido, formamidoalkyl, aryl, alkylaryl, alkoxyaryl, hydroxyalkylaryl, alkoxyalkylaryl, hydroxyalkyloxyaryl, alkoxyalkyloxyaryl, alkylthioaryl, hydroxyalkylthioaryl, hydroxyalkylthioalkylaryl, hydroxyalkylthioalkyloxyaryl, aralkyl, aralkyloxy, alkoxyaralkyl, alkoxyaralkyloxy, aryloxy, alkylarylaxy, alkoxyaryloxy, and heterocyclyl; each Q is independently selected from hydrogen, alkyl, hydroxyl, carboxyl, carboxyalkyl, hydroxyalkyl, alkylthioalkyl, sulfhydryl, thioalkyl, alkoxy, alkylthio, alkylamino, aminoalkyl, aminoalkylaminoalkyl, hydroxy-alkylaminoalkyl, hydroxylaminoalkyl, hydroxamido, formamidoalkyl, alkylformamido, aryl, including substituted aryl, aryloxy, heterocyclyl, carbonyliminodiacetic acid, methyle eiminodiacetic acid, methylenethioethylene-iminodiacetic acid, carboxyalkylthioalkyl, a residue of ethylenediaminetetraascetic acid (EDTA), a residue of diethylenetriaminepentaacetic acid (DTPA), hydrazinylidenediacetic acid, and a salt of any of the foregoing acids; each Z is independently selected from a heteroatom with a valence of two, a heteroatom with a valence of three, an alkylene group, an alkylene group bonded to a heteroatom having a valence of two, and an alkylene group bonded to a heteroatom having a valence of three; each X is independently selected from nitrogen and a residue of an alkylene group; each W is independently selected from hydrogen and a substituent that comprises a protein reactive group; each L' is independently selected from a chemical bond and an intra-ring linking group; each L is independently selected from a residue of an alkylene group and an extra-ring linking group; n is 1, 2, 3 or 4; and each p is independently 0, 1, 2, 3 or 4; provided that only one W is a protein reactive group; the L bonded to the W that is a protein reactive group contains 1, 2, or 3 carbon atoms and connects X to a heteroatom capable of participating in the chelation of a metal ion; and when X is a nitrogen and a heteroatom of Z is bonded to X, the heteroatom of Z is also nitrogen; and the immunoreactive group is an ST receptor binding moiety.

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TARGETING IMMUNOREAGENTS USEFUL IN THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS AND METHODS

FIELD OF THE INVENTION

The present invention relates to targeting radioactive immunoreagents which find particular utility in therapeutic and diagnostic imaging compositions and methods.

BACKGROUND OF THE INVENTION

A comprehensive discussion of the background to the present invention may be found in US Patent No. 5760191.

In summary, the various commercially available radiolabeled antibod es and chelating agents employed for making immunoreactive conjugates by covalently bonding a chelating agent to the immunoreactive protein, as well as radionuclide complexes thereof for use in diagnostic imaging and targeted therapeutics, suffer from one or more of the following disadvantages: 1) destruction or excretion of the reagent due to rapid catabolism or metabolism; 2) inefficient covalent bonding of the radioactive component with protein in conjugate preparation; 3) slow complexation with metals; 4) unstable metal complexation, e.g., with respect to temperature, time or pH 5) inability to form conjugates and remain stable in storage until metal complexation is desired; 6) inability ao spectro-photometrically analyze the radionuclide complex reagent; and 7) inability to complex without activation steps that degrade protein.

In WO92/08494, co-workers have disclosed targeting radioactive immunoreagents which effectively solve most of the problems of the prior art discussed above. The targeting radioactive immunoreagents of that patent application comprise a metal radionuclide ion, a complexing agent which is a derivative of a pyridine,

bipyridine, terpyridine, quaterpyridine, quinquepyridine, sexipyridine or phenanthroline, and an immunoreactive group covalently bonded through a protein reactive group to the complexing agent.

While a significant advance over the prior art, the chelators of WO 92/08494, particularly the macrocyclic oligo-2,6-pyridyl moieties therein, by virtue of the protein reactive group being attached either directly to a pyridine ring at a 3-, 4- or 5- position of the ring or, indirectly, to a group that is attached to a pyridine ring at a 3-, 4- or 5- position of said ring, may have substituent electron density donating or electron density withdrawing properties that affect electron distribution at the pyridyl chelating site in a metal complex. Similarly, in immunoconjugates comprising chelators of WO 92/08484, the attachment of an immunoreactive group by reaction of a reactive group on the immunoreactive moiety with the protein reactive group results in a change in the electron configuration in (i.e., a change in the chemical bonds of) the protein reactive group, such as occurs, for example, in a change from an isothiocyanate group to a thiourea group during reaction with an amine group on an immunoreactive group such as an antibody. This change in the nature of the substituent attached either directly to a pyridine ring at a 3-, 4- or 5-position of the ring or, indirectly, to a group that is attached to a pyridine ring at a 3-, 4- or 5-position of said ring, c n change the electron distribution at the chelating site of the pyridine ring and can thus affect the metal binding and spectroscopic properties of the chelate. Thus, it would be desi able provide chelators that retain the advantages of the oligo-2,6-pyridyl chelators of WO 92/08494, the pyridine groups of which are not subject to alterations-in electron density caused by reaction of the protein reactive group.

In addition, for metal binding and spectroscopic

properties of the macrocyclic chelating agents such as those described in WO 92/08494 and of metal complexes thereof to be only minimally subject to changes in the microenvironment experienced by the chelating site, such as would occur when a protein reactive group reacts with a reactive site on a protein and which results in the protein occupying space that would otherwise be occupied by solvent molecules such as water, (i.e., in order to enhance the stability of the metal chelate) it is desirable that groups peripheral to the macrocyclic oligo-2,6-pyridine moiety have the ability to coordinate with or supply electron density to the metal ion and thus mask the effects of a change in microenvironment. Such coordination can be either through a primary coordination array of ligand electron density such as obtains when ligands are directly bonded to available metal ion (for example, at octahedral geometric sites of metal ion coordination), or through an outer, secondary array of coordination electron density which is further removed than the primary coordination array from, but still interacts with, the metal ion.

This invention provides oligo-2,6-pyridinyl containing targeting immunoreagents having a protein reactive group attached to the complexing agent at a position other than directly to the 3-, 4-, or 5- - position of a pyrifine ring of the oligo-2,6-pyridyl containing component or indirectly through a substituent to a 3-, 4-, or 5- position of a pyridine ring of the oligo-2,6-pyridyl-containing componenc.

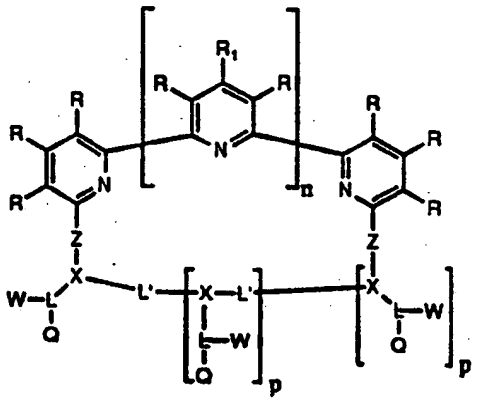
This invention further provides such oligo-2,6-pyridinyl-containing targeting immunoreagents wherein the protein reactive group is attached to the complexing agent by a linking group comprising a group of 1, 2, or 3 carbon atoms attached to the macrocyclic ring, at least one carbon atom of which is attached to a heteroatom which can participate in the chelation of a metal ion chelated by the oligo-2,6-pyridinyl-containing

macrocyclic portion of the chelating agent.

Such complexing (or chelating) agents of the targeting immunoreagent have the advantage that they do not require chemical modification directly at a 3-, 4-, or 5-position of a pyridine ring of the oligo-2,6-pyridine moiety or indirectly at a substituent at a 3-, 4-, or 5-position of a pyridine ring of the oligo-2,6-pyridine moiety to introduce a protein reactive group, which modification can otherwise cause a perturbation of the electron distribution at the pyridyl chelating site and which perturbation can change as a result of the protein reactive group reacting with a protein. Another advantage is that the chelating ability of the oligo-2,6-pyridine moiety of the macrocylic chelator can be modified by appropriate introduction of non-protein reactive group substituents at one or more of the 3-, 4-, or 5-positions of the pyridine rings of the oligo-e,6 pyridine moiety. The effect of this modification is not changed when a protein reactive group in another part of the molecule reacts with a protein.

Summary of the Invention

The present invention thus provides a targeting immunoreagent comprising a metal ion, a residue of a complexing agent and a immunoreactive group linked to said complexing agent having the structure.



Structure I

wherein

each R and R_1 is independently selected from hydrogen, alkyl, alkoxy, hydroxyalkyl, alkoxyalkyl, hydroxyalkyloxy, alkoxyalkyloxy, alkylthio, alkylthioalkyl, alkylthioalkyloxy, hydroxyalkylthio, hydroxyalkylthioalkyl, hydroxyalkylthioalkyloxy, N,N--dialkylamino, N-(hydroxyalkyl)-N-alkylamino, N,N--bis(hydroxyalkyl)amino, N,N-dialkylaminoalkyl, N--(hydroxyalkyl)-N-alkylaminoalkyl, N,N-bis(hydroxyalkyloaminoalkyl, alkylformamido, formamidoalkyl, aryl, alkylaryl, alkoxyaryl, hydroxyalkylaryl, alkoxyalkylaryl, hydroxyalkyloxyaryl, alkoxyalkyloxyaryl, alkylthioaryl, hydroxyalkylthioaryl, hydroxyalkylthioalkylaryl, hydroxyalkylthioalkyloxyaryl, aralkyl, aralkyloxy, alkoxyaralkyl, alkoxyaralkyloxy, aryloxy, alkylarylaxy, alkoxyaryloxy, and heterocyclyl;

each Q is independently selected from hydrogen, alkyl, hydroxyl, carboxyl, carboxyalkyl, hydroxyalkyl, alkylthioalkyl, sulfhydryl, thioalkyl, alkoxy, alkylthio, alkylamino, aminoalkyl, aminoalkylaminoalkyl, hydroxy-alkylaminoalkyl, hydroxylaminoalkyl, hydroxamido, formamidoalkyl, alkylformamido, aryl, including substituted aryl, aryloxy, heterocyclyl, carbonyliminodiacetic acid, methyle eiminodiacetic acid, methylenethioethylene-iminodiacetic acid, carboxyalkylthioalkyl, a residue of ethylenediaminetetraacetic acid (EDTA), a residue of diethylenetriaminepentaacetic acid (DTPA), hydrazinylidenediacetic acid, and a salt of any of the foregoing acids;

each Z is independently selected from a heteroatom with a valence of two, a heteroatom with a valence of three, an alkylene group, an alkylene group bonded to a heteroatom having a valence of two, and an alkylene group bonded to a heteroatom having a valence of three;

each X is independently selected from nitrogen and a residue of an alkylene group;

each W is independently selected from hydrogen and a substituent that comprises a protein reactive group;

each L' is independently selected from a chemical bond and an intra-ring linking group;

each L is independently selected from a residue of an alkylene group and an extra-ring inking group;

n is 1, 2, 3 or 4; and

each p is independently 0, 1, 2, 3 or 4;

provided that only one W is a protein reactive group; the L bonded to the W that is a protein reactive group contains 1, 2, or 3 carbon atoms and connects X to a heteroatom capable of participating in the chelation of a metal ion; and when X is a nitrogen and a heteroatom of Z is bonded to X, the heteroatom of Z is also nitrogen;

and the immunoreactive group is an ST receptor binding moiety.

In another embodiment, this invention provides a targeting immunoreagent e.g. a targeting radioactive, paramagnetic or fluorescent immunoreagent comprising a metal ion, e.g. a radionuclide ion, paramagnetic metal ion or fluorescent metal ion, a complexing agent, and an immunoreactive group attached through a linking group to said complexing agent, wherein the complexing agent has the structure I as defined above and the linking group between the complexing agent and the immunoreactive group comprises the residue of the protein reactive group on the complexing agent.

This invention also provides therapeutic and diagnostic compositions comprising the above-described targeting immunoreagents.

This invention further provides a method for diagnostic imaging a site in a patient comprising a) administerinn to the patient an effective amount of the above-described targeting immunoreagent capable of targeting the site in a pharmaceutically acceptable carrier therefor, and i) imagewise activating a

radiation-sensitive element or device, such as, for example, a film or electronic sensor, with the radiation emitted from the targeted site or;

ii) imagewise activating a nuclear magnetic resonance detection sensor element or device which is sensitive to a change in one or more nuclear magnetic relaxation properties of an isotope such as a proton at the site of the patient while exposed to a controlled magnetic field environment such as, for example, a magnetic field in a magnetac resonance imaging instrument, which change is induced by a paramagnetic metal ion of the immunoreagent, or;

iii) irradiating the specimen with light, and imagewise activating a fluorescence emission sensor element or device, such as, for example, a film or electronic sensor, with the fluorescent light emitted from the targeted site. A preferred method uses time delayed fluorescence detection.

This invention further provides a method for treating a disease sise in a patient comprising administering to the patient or a specimen from the patient an effective amount of a therapeutic composition comprising the above-described radioactive immunoreagent capable of targeting the site and a pharmaceutically acceptable carrier therefor.

It is an advantageous feature of this invention that the targeting immunoreagents of this invention are not rapidly metabolized and do not deleteriously disperse.

It is another advantageous feature that the complexing agents efficiently attach to proteins and other biological molecules.

Yet another advantageous feature of this invention is that the immunoreagents exhibit photometric emissions which have a low signal to noise ratio, good energy emission characteristics, and which are readily subject to spectrophotometric analysis.

Additionally, protein conjugates of the complexing agents can be formed and stored until metal complexation is desired, and complexation can be accomplished without activation steps that degrade protein.

Moreover, the complexing agents rapidly complex with metals, and the resulting chelates exhibit excellent stability with respect to time, temperature and pH.

Brief Description of the Drawings

Figure 1 relates to TMT-ST. The mass spectroscopy of which can be found in Figure 2.

Figure 2 relates to the mass spectrum of the TMT-ST conjugate prepared in Example 57. The $(M + H)^+$ peak at 26(7.0 and the $(M + 2H)^{++}$ peak at 1328.6 clearly demonstrate that TMT-ST has been formed, rather than the expected TMT-Gly-ST. This can be explained if either the TMT-NCS is adsorbed to the resin non-covalently in the TMT-Gly-Oxime Resin above, or the TMT thiourea bond is more electrophilic than the glycine oxime ester.

Description of Preferred Embodiments

The description which follows primarily concerns the targeting immunoreagents of the invention, as well as usage of the targeting immunoreagents of the invention in therapeutic and diagnostic imaging compositions and methods. In addition, targeting radioactive immunoreagents of the invention are useful as diagnostic reagents, for example, as radioimmunoelectro-phoresis reagents.

The complexing agents of use in the targeting immunoreagents of the invention comprise a macrocyclic oligo-2,6-pyridine-containing ring which is a derivative of a terpyridine, or a quaterpyridine, or a quinquepyridine or a sexipyridine and which has the structural formula I recited in the Summary above.

Each R and R_1 in formula I independently is selected

from: hydrogen; straight or branched chain or cyclic saturated alkyl, preferably containing from 1 to about 20 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, s-butyl, t-butyl, 2-ethylhexyl, decyl, hexadecyl, octadecyl, cyclohexyl, cyclopropyl, etc.;

alkoxy, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl;

hydroxyalkyl, the alkylene portion of which is a straight or branched chain or cyclic alkylene group, preferably containing from 1 to about 20 carbon atoms, such as methylene, ethylene, propylene, isopropylene, butylene, s-butylene, t-butylene, 2-ethylhexylene, decylene,

hexadecylene, octadecylene, cyclohexylene, cyclohexanedimethylene, cyclopropylene, etc.;

alkoxyalkyl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the alkylene portion of which is a straight or branched chain or cyclic alkylene group which contains from 1 to about 20 carbon atoms as described above for alkylene;

hydroxyalkylaxy, the alkylene portion of which is a straight or branched chain or cyclic alkylene group which contains from 2 to about 20 carbon atoms as described above for alkylene, and the oxygen atoms of which are separated by at least two carbon atoms;

alkoxyalkyloxy, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, and the oxygen atoms of which are separated by at least two carbon atoms;

alkylthio, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl;

alkylthioalkyl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the alkylene portion of which is a residue of an alkyl group which contains from 1 to about 20 carbon atoms as described above for alkylene;

alkylthioalkyloxy, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, and the oxygen and sulfur atoms of which are separated by at least two carbon atoms;

hydroxyalkylthio, the alkylene portion of which contains from 2 to about 20 carbon atoms as described above

for alkylene, and the oxygen and sulfur atoms of which are separated by at least two carbon atoms;

hydroxyalkylthioalkyl, the alkylene of the hydroxyalkyl portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, the sulfur and oxygen atoms of which are separated by at least two carbon atoms, and the alkylene of the thioalkyl portion of which independently contains from 1 to about 20 carbon atoms as described above for alkylene;

hydroxyalkylthioalkyloxy, the alkylene portions of which independently contain from 2 to about 20 carbon atoms as described above for alkylene and the sulfur and oxygen atoms of which are separated by at least two carbon atoms;

N,N-dialkylamino, each alkyl portion of which independently contains from 1 to about 20 carbon atoms as described above for alkyl;

N-hydroxyalkyl-N-alkylamino, the alkylene of the N-hydroxyalkyl portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen and nitrogen atoms of which are separated by at least two carbon atoms, and the N-alkyl portion of which contains from 1 to about 20 carbon atoms as described for alkyl above;

N,N-bis(hydroxyalkyl)amino, the alkylene of each N-hydroxyalkyl portion of which contains from 2 to about 20 carbon atoms as described above for alkylene and the oxygen and nitrogen atoms of which are separated by at least two carbon atoms;

N,N-dialkylaminoalkyl, the alkyl of each of the N,N-alkyl portions of which independently contains from 1 to about 20 carbon atoms as described above for alkyl, and the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene;

N-hydroxyalkyl-N-alkylaminoalkyl, the alkylene of

the N-hydroxyalkyl portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen and nitrogen atoms of which are separated by at least two carbon atoms, the alkyl of the N-alkylamino portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene;

N,N-bis(hydroxyalkyl)aminoalkyl, the alkylene of each hydroxyalkyl portion of which independently contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen and nitrogen atoms of which are separated by at least two carbon atoms, and the alkylene-of the aminoalkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkylene;

alkylformamido, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl;

formamidoalkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene;

unsubstituted and substituted aryl, the aryl portion of which preferably contains from about 6 to 24 carbon atoms, such as phenyl, naphthyl, and phenanthryl, and the substituents of which are preferably selected from alkyl, nitro, halogen (such as chloro, bromo, and iodo), N,N-dialkylamino as defined above, alkoxy as defined above, alkylthio as defined above, carboxy, sulfonato, hydroxyalkyl as defined above, and alkoxyalkyl as defined above, for example, nitrophenyl, chlorophenyl, phenoxyphenyl, N-hexadecyl-N-methylaminophenyl, N-methyl-N-octadecyl-aminophenyl, 4-methoxy-3-iodophenyl, 4-methoxy-3-(N,N-dimethyl)phenyl, methylthiophenyl, carboxyphenyl, and sulfonatophenyl;

alkylaryl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the arylene portion of which is the residue of an aryl group containing from 6 to 24 carbon atoms as described above, such as alkylphenyl, for example, tolyl, xylyl and ethylphenyl;

alkoxylaryl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, methoxyphenyl, methylenedioxyphenyl, methoxyethoxyphenyl, and dimethoxyphenyl;

hydroxyalkylaryl, the alkylene of which contains from 1 to about 20 carbon atoms as described above for alkylene and the arylene portion of which contains from 6 to about 24 carbon atoms as described for arylene above, for example, hydroxyethylphenyl, bis(hydroxymethyl)phenyl, dihydroxycyclohexylphenyl, and hydroxymethylanthracenyl;

alkoxyalkylaryl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene, and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, 4-(2-methoxyethoxy)ethylphenyl, t-butoxypropylnaphthyl, and 2-(2,3-dimethoxypropoxy)ethylphenyl;

hydroxyalkyloxyaryl, the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen atoms of which are separated by at least two carbon atoms, and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, 4-(2-hydroxyethoxy)phenyl, and 5-hydroxypropoxy-3,4-methylenedioxyphenyl;

alkoxyalkyloxyaryl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen atoms of which are separated by at least two carbon atoms, and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, 4-(2-ethoxyethoxy)phenyl, methoxyethoxynaphthyl, methoxyethoxyphenyl, 3,4-bis(2-methoxyethoxy)phenyl, and 4-poly(ethylene oxidyl)phenyl, the poly(ethylene oxidyl) portion of which contains from 2 to 100 recurring units of ethylene oxide;

alkylthioaryl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, methylthiophenyl, and 4-(2-ethylthio)phenyl;

hydroxyalkylthioaryl, the alkylene portion of which contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen and sulfur atoms of which are separated by at least two carbon atoms, and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, 2-hydroxyethylthiophenyl, 2,3-dihydroxypropylthiophenyl, and 4-(2,3-dihydroxypropyl)thio-3-methoxyphenyl;

hydroxyalkylthioalkylaryl, the hydroxyalkylthio- portion of which contains an alkylene group having from 2 to about 20 carbon atoms as described above for alkylene, the oxygen and sulfur atoms of which are separated by at least two carbon atoms, the alkylene of the thioalkylaryl portion of which contains from 1 to about 20 carbon atoms as described above for alkylene, and the arylene portion of which contains from 6 to about 24 carbon atoms as described

above for arylene, for example, (2-hydroxyethyl)thiomethylphenyl, 2-(2,3-dihydroxypropyl)thioethylphenyl, and 4-[(2,3-dihydroxypropyl)thiomethyl]phenyl;

hydroxyalkylthioalkylaxyaryl, each alkylene portion of which independently contains from 2 to about 20 carbon atoms as described above for alkylene, the oxygen and sulfur atoms of which are separated by at least two carbon atoms, and the arylene portion of which contains from 6 to about 24 carbon atoms as described above for arylene, for example, 4-[2-(2-hydroxyethyl)thioethoxy]phenyl, and 4,5-bis[2-(2-hydroxyethyl)thioethoxy]naphthyl;

aralkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene and the aryl portion of which contains from about 6 to 24 carbon atoms as described above for aryl, for example, benzyl, trimethylbenzyl; and 9-(10-methyl)anthracenyl;

aralkyloxy, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene, and the aryl portion of which contains from about 6 to 24 carbon atoms as described above for aryl, for example, benzyloxy, methylenedioxybenzyloxy, 2-(methylenedioxyphenyl)ethoxy and phenylethoxy;

alkoxyaralkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described for alkylene above, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the arylene portion of which contains from about 6 to 24 carbon atoms as described above for arylene, for example, 3-(2,3-dimethoxypropoxy)benzyl, methoxybenzyl, and 4-poly(ethylene oxidyl)benzyl, the poly(ethylene oxidyl) portion of which contains from 2 to 100 recurring units of ethylene oxide;

alkoxyaralkyloxy, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene, and the arylene portion of which contains from about 6 to 24 carbon atoms as described above for arylene, for example, 3-(2,3-dimethoxypropoxy)benzylaxy, methoxybenzyloxy, and 4-poly(ethylene oxidyl)benzyloxy, the poly(ethylene oxidyl) portion of which contains from 2 to 100 recurring units of ethylene oxide;

aryloxy, the aryl portion of which contains from 6 to about 24 carbon atoms as described above for aryl, such as phenoxy, nitrophenoxy, bromophenoxy, carboxyphenoxy, and sulfonatophenoxy;

alkylaryloxy, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the arylene portion of which contains from about 6 to 24 carbon atoms as described above for arylene, for example, methylphenoxy, cyclohexylphenoxy, and butylphenoxy;

alkoxyaryloxy, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl, and the arylene portion of which contains from about 6 to 24 carbon atoms as described above for arylene, for example, methoxyphenoxy and poly(ethylene oxidyl)phenoxy, the poly(ethylene oxidyl) portion of which contains from 2 to 100 recurring units of ethylene oxide; and

substituted or unsubstituted heterocyclyl, containing from 5 to about 36 total nuclear carbon and heteroatoms and preferably comprising one ore more rings comprised of 5 or 6 nuclear carbon and heteroatoms such as N, S, P or O, for example, pyridyl, methylpyridyl, N-morpholino, dimethylaminopyridyl, methoxypropylpyridyl,

oxazolyl, imidazolyl, pyrazolyl, quinolyl, thiazinyl, furanyl, pyranyl, and dimethylphosphazinyl.

In presently especially preferred embodiments, R is hydrogen and R_1 is a phenyl or a 4-alkoxyphenyl group.

Each Q in the above formula is independently selected from hydrogen, alkyl, alkoxy, hydroxyalkyl, alkylthioalkyl, alkylthio, alkylamino, hydroxyalkyl-aminoalkyl, formamidoalkyl, alkylformamido, aryl, including substituted aryl, aryloxy, and heterocyclyl, each of the foregoing preferably being as defined above for R and R_1; hydroxamido; hydroxylaminoalkyl, the alkyl portion of which contains from 1 to about 20 carbon atoms as described above for alkyl; aminoalkylaminoalkyl, the alkylene of the aminoalkylamino portion of which contains from 2 to about 20 carbon atoms as described above for alkylene and the other alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene; aminoalkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene; thioalkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene; sulfhydryl; hydroxyl; carboxyl; carboxyalkyl, the alkylene portion of which contains from 1 to about 20 carbon atoms as described above for alkylene; carboxyalkylthioalkyl, the alkylene portions of which contains from 1 to about 20 carbon atoms as described above for alkylene; carbonyliminodiacetic acid; methyleneiminodiacetic acid; methylenethioethylene-iminodiacetic acid; hydrazinylidenediacetic acid; a residue of ethylenediaminetetraacetic acid (EDTA); a residue of diethylenetriaminepentaacetic acid (DTPA); and a salt of any of the foregoing acids.

Each Z in Structure I is independently selected from a heteroatom with a valence of two, such as oxygen and

sulfur; a heteroatom with a valence of three, such as nitrogen, an alkylene group containing 1 to 20 carbon atoms, for example, methylene, ethylene, propylene, isopropylene, isobutylene, etc.; an alkylene group containing 1 to 20 carbon atoms as described above bonded to a heteroatom having a valence of two, such as oxygen and sulfur; and an alkylene group containing 1 to 20 carbon atoms as described above bonded to a heteroatom having a valence of three such as nitrogen.

Each X in Structure I is independently selected from nitrogen and a residue of an alkylene group containing 1 to 20 carbon atoms, for example, methylene, ethylene, propylene, isopropylene, isobutylene, etc.

The term "residue" is used herein in the context of a chemical entity comprising, for example, a ligand, or an alkyl group, or a chelating group, or a radioactive agent, or a linking group, or a protein reactive group, or an

immunoreactive group, or an immunoreactive material, or an immunoreactive protein, or an antibody, or an antibody fragment, or a crosslinking agent such as a heterobifunctional crosslinking agent and is defined as that portion of a chemical entity which exclusively remains when one or more chemical bonds of which the chemical entity is otherwise comprised when considered as an independent chemical entity, is altered, modified, or replaced to comprise one or more covalent bonds to one or more other chemical entities. For example, in one aspect, a linking group between an immunoreactive group and a chelating agent comprises the residue of a protein reactive group of the chelating agent and the residue of the reactive group on the immunoreactive group with which the protein reactive group reacted. In this regard, when a protein reactive group such as an isothiocyanato group (i.e., an -N=C=S) on a chelating agent reacts with a reactive group such as an amine group (i.e., an H_2N-) on an immunoreactive group to form a thioureylene group [i.e., an -NH-C(=S)-HN-] linking the chelating agent with the immunoreactive group, the thioureylene group is a linking group comprising the residue of the protein reactive group and the residue of the amine group.

L' in Structure I refers to a chemical bond or a divalent "intra-ring linking group", one valence of which is attached to an X and the other valence of which is attached to either another X or to a Z. As such, when either valence of L' is attached to an alkylene group, L' can be a chemical bond, an alkylene group of 1 to 10 carbon atoms as described above for an alkylene group of R and R_1, or a part of an arylene group of 6 to 20 carbon atoms such as, for example, phenylene and others as described above for an arylene group of R and R_1 above. In addition, the alkylene group can be

interrupted with one or more heteroatoms selected from oxygen, sulfur, and selenium, such as, for example, oxygen in ethyleneoxyethylene, sulfur in ethylenethioethylene, ethylenethio, thioethylene, ethylenethioethylenethio and ethylenedithioethylene, and selenium as ethyleneseleno-ethylene, or with heteroatom-containing groups such as carbonyl, sulfonyl and sulfinyl. The alkylene group can also be interrupted with a substituted or unsubstituted heterocyclic group, preferably containing rings comprised of 5 or 6 nuclear carbon and heteroatoms such as N, S, Se, P or O, for example, pyridyl, methylpyridyl, (N-carboxy-methyl)morpholino, dimethylaminopyridyl, methoxypropyl-pyridyl, oxazolyl, imidazolyl, pyrazolyl, quinolyl, thiazinyl, furanyl, pyranyl, and methylphosphazinyl.

When both valences of L' are attached to heteroatoms, L' can be a chemical bond which links two nitrogen atoms, an alkylene group of 2 to 10 carbon atoms as described for an alkylene group of R and R_1 above, or a part of an arylene group of 6 to 20 carbon atoms such as, for example, phenylene and others as described for an arylene group of R and R_1 above. In addition, the alkylene group can be interrupted with one or more heteroatoms selected from oxygen, sulfur, and selenium, such as, for example, oxygen in ethyleneoxyethylene, sulfur in ethylenethio-ethylene, ethylenethio, thioethylene, ethylenethio-ethylenethio and ethylenedithioethylene, and selenium in ethyleneselenoethylene, or with heteroatom-containing groups such as carbonyl, sulfonyl and sulfinyl. The alkylene group can also be interrupted with a substituted or unsubstituted heterocyclylic group, preferably containing rings comprised of 5 or 6 nuclear carbon and heteroatoms such as N, S, Se, P or O, for example, pyridyl, methylpyridyl, (N-carboxy-methyl)morpholino, dimethylaminopyridyl, methoxy-

propylpyridyl, oxazolyl, imidazolyl, pyrazolyl, quinolyl, thiazinyl, furanyl, pyranyl, and methylphosphazinyl.

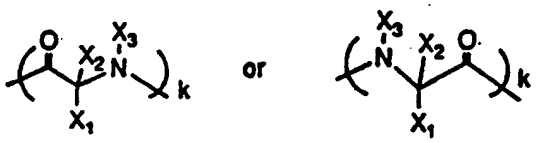
L in Structure I refers to a residue of an alkylene group or to a trivalent "extra-ring linking group", one valence of which is attached to an X, one valence of which is attached to a W, and the third valence of which is attached to a Q. When W is a protein reactive group, X is connected by L to a heteroatom such as oxygen, nitrogen, or sulfur, the oxygen of which is in an ether, ester, carbonyl, sulfoxyl, sulfonyl, phosphonyl, sulfonate, phosphate, or carboxyate group, the nitrogen of which is in an amide, amine, hydroxylamine, hydrazine, urea, thiourea, nitrile or imine group, and the sulfur of which is in a sulfhydryl, thioether, thiocarbonyl or disulfide group, which heteroatom is capable of participating in the chelation of a metal ion, and wherein L is a group that contains 1, 2, or 3 carbon atoms such as, for example, the residue of an alkylene group containing from 1 to 3 linearly bonded carbon atoms, i.e., methylene, ethylene, and propylene, and such as, for example, the residue of groups such as carbonylmethyl, ethyloxy, propyloxy, ethylamido, ethylthio, ethylamino, and propylthio. In addition, when L is ethylene or propylene, from 2 to about 100 of such ethylene groups, propylene groups, or combinations of ethylene and propylene groups can be tandemly linked by heteroatoms, each linking heteroatom being independently selected from oxygen, sulfur, and selenium, such as, for example, oxygen in ethyleneoxy-ethylene, propyleneoxypropylene, ethyleneoxypropylene, poly(ethyleneoxy)ethylene wherein the polymer contains from 2 to about 100 ethylene units, poly(propyleneoxy)propylene wherein the polymer contains from 2 to about 100 propylene units, poly(ethyleneoxy-copropyleneoxy)ethylene, poly(ethyleneoxy-copropyleneoxy)propylene,

poly(propyleneoxy-co-ethyleneoxy)ethylene, and poly(propyleneoxy-co-ethyleneoxy)propylene wherein each polymer contains from 2 to about 100 ethylene and propylene units, sulfur in ethylenethioethylene, ethylenethio-propylene, propylenethioethylene, ethylenethioethylene-thioethylene, and ethylenedithioethylene, and selenium in ethyleneselenoethylene and propyleneselenopropylene, or combinations such as ethyleneoxyethylenethioethylene, propyleneoxyethyleneselenoethylene, and the like.

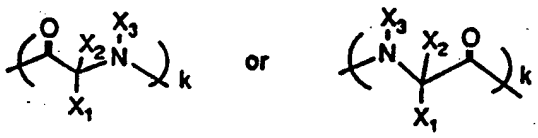
Furthermore, from 2 to about 100 of such ethylene groups, propylene groups, or combinations of ethylene and propylene groups can be tandemly linked by combinations of heteroatom linking groups as described above and heteroatom-containing linking groups such as carbonyl, sulfonyl and sulfinyl, oxycarbonyl, and carbonyloxy. In addition, from 2 to about 100 of such ethylene groups, propylene groups, or combinations of ethylene and propylene groups can be tandemly linked by combinations of heteroatom linking groups as described above, heteroatom-containing linking groups as described above, and substituted or unsubstituted heterocyclic linking groups, which heterocyclic linking groups preferably contain rings comprised of 5 or 6 nuclear carbon and heteroatoms such as N, S, Se, P or O, for example, pyridylene, methylpyridylene, morpholinoene, dimethylaminopyridylene, methoxypropyl-pyridylene, oxazolylene, imidazolylene, pyrazolylene, quinolylene, thiazinylene, furanylene, pyranylene, and methylphosphazinylene.

The trivalent extra-ring linking group of L can be selected from a nitrogen atom; a nitrogen atom covalently linked to a methylene, ethylene or propylene group or combinations of ethylene and propylene groups as described

for residues of the alkylene group of L above; an amino acid linkage, i.e., a



group wherein k=1 and X_1 and X_2 comprise the components of a naturally occurring amino acid or the optical enantiomer thereof such as alanine, glycine, serine, lysine, tyrosine, phenylalanine, glutamic acid, aspartic acid, and the like and X_3 is as described below, or X_1, X_2, X_3 independently are H, alkyl containing from 1 to 10, preferably 1 to 6, carbon atoms such as methyl, ethyl and propyl, and methylene, ethylene or propylene groups or combinations of ethylene or propylene groups as described for residue of the alkylene group of above; or a peptide linkage, i.e., a



group wherein k>1 and each X independently is represented by a group as described for X_1, X_2, X_3 above. Especially preferred extra-ring linking groups include the residues of ethylene and propylene groups as described above.

As used herein, "protein reactive group" refers to a group W in Structure I which can react with a reactive functional group typically found on or introduced into a protein, especially an immunoreactive protein, to form a linking group between the complexing agent and the protein. However, it is specifically contemplated that a protein reactive group can be used to conjugate a complexing agent

of this invention to a non-protein biomolecule as well as to a non-biological molecule such as a synthetic chemical substance (for example, a drug) that is of interest, for example, for the purposes of detection of such a molecule in a mixture which may contain such a synthetic chemical substance and which substance contains a group that is reactive with the protein reactive group. Thus, the protein reactive groups useful in the practice of this invention include those groups which can react with any molecule, preferably a biological molecule (such as a protein, a carbohydrate, a nucleic acid, and a lipid) containing a reactive group to form a linking group between the complexing agent and the molecule. Preferably the molecule is a protein, and preferred reactive groups on such protein molecule include amine groups and sulfhydryl groups. Especially preferred biological molecules contain an immunoreactive group as described hereinbelow.

The protein reactive groups useful in the practice of this invention also include those groups which can react with any biological molecule that is chemically modified, for example, by oxidation, by reduction, or by covalent bond formation such as by amide bond formation with another chemical species such as, for example, an amine, an amino acid, a substituted amine, or a substituted amino acid, to introduce a reactive group into the biological molecule, to form a linking group between the complexing agent and the chemically modified biological molecule.

The protein reactive groups useful in the practice of this invention also include those groups which comprise a portion of a specific receptor-ligand interactive group. For example, in the complexing agent of structure I, W can comprise an oligonucleotide group as a receptor portion of a receptor-ligand interactive group. The complementary

oligonucleotide attached to a biological molecule is then a ligand portion of the receptor-ligand interactive group. Said ligand will bind to the receptor to form a linking group between the complexing agent and the biological molecule.

Preferred protein reactive groups can be selected from, but are not limited to, groups that will react directly with an amine group such as a lysine epsilon amine group or a terminal amine group in a peptide or with a sulfhydryl group such as a cysteine sulfhydryl group commonly found on a protein or other biological molecule. Examples of such protein reactive groups include active halogen-containing groups such as chloromethylphenyl groups, chloromethylcarbonyl groups, and iodomethylcarbonyl groups; activated 2-leaving-group substituted ethylsulfonyl and ethylcarbonyl groups such as 2-chloroethylsulfonyl groups and 2-chloroethylcarbonyl groups; vinylsulfonyl groups; vinylcarbonyl groups; oxiranyl groups; isocyanato groups; isothiocyanato groups; aldehydo groups; aziridyl groups; succinimidoxycarbonyl groups; activated acyl groups such as carboxylic acid halide groups; anhydride groups; thioester groups; carbonates such as nitrophenylcarbonates; sulfonic acid esters; phosphoramidates; cyanuric monochlorides and cyanuric dichlorides; and other groups known to be useful in conventional photographic gelatin hardening agents.

The above listed protein reactive groups can react with a protein or other biological molecule which is chemically modified to contain reactive amine groups and sulfhydryl groups. Amine groups can be introduced by well known techniques such as, for example, nitration of a phenyl group followed by reduction, by conversion of a primary amide to an amine with nitrous acid, by conversion of a hydroxyl group of an alcohol into a sulfonic acid ester

followed by displacement with an azide group and subsequent reduction to an amine, and the like. Sulfhydryl groups can be introduced by well known techniques such as, for example, by conversion of a hydroxyl group of an alcohol into a sulfonic acid ester followed by displacement with sodium sulfide, by dehydrative amide bond formation between an amine group of a protein and a carboxylic acid group of an acetylated cysteine using a carbodiimide reagent followed by treatment with hydroxylamine, and the like.

In addition, when a protein or other biological molecule can be chemically modified such as by partial oxidation to introduce an aldehyde group or a carboxylic acid group, a preferred "protein reactive group" can be selected from amino, amincalkyl, aminoaryl, alkylamino, arylamino, hydrazino, alkylhydrazino, arylhydrazino, carbazido, semicarbazido, thiocarbazido, thiosemicarbazido, sulfhydryl, sulfhydrylalkyl, sulfhydrylaryl, hydroxy, carboxy, carboxyalkyl and carboxyaryl. The alkyl portions of the protein reactive group can contain from 1 to about 20 carbon atoms as described for R and R_1 above, and the aryl portions of the protein reactive group can contain from about 6 to about 24 carbon atoms as described for R and R_1 above.

An additional preferred protein reactive group can comprise a residue of a crosslinking agent. A useful crosslinking agent can react with a functional group such as, for example, an amine or sulfhydryl or carboxylic acid group or aldehyde group found in W of Structure I above and with a functional group such as, for example, an amine or sulfhydryl or carboxylic acid group or aldehyde group found in a protein or a biological molecule or in a chemically modified protein or biological molecule such as described above. The residues of certain useful crosslinking agents,

such as, for example, difunctional gelatin hardeners, bisepoxides and bisisocyanates become a part of, i.e., a linking group in, a protein-complexing agent conjugate or a biological molecule-complexing agent conjugate which is formed as a result of the crosslinking reaction of such a crosslinking protein reactive group with a complexing agent and also with a protein or also with a biological molecule, respectively.

Other useful crosslinking agents, however, facilitate the crosslinking, for example, as consumable catalysts, and are not present in the final conjugate. Examples of such crosslinking agents are carbodiimide and carbamoylonium crosslinking agents as disclosed in U.S. Patent 4,421,847, the disclosure of which is hereby incorporated herein by reference in its entirety, and the dication ethers of U.S. Patent 4,877,724, the disclosure of which is hereby incorporated herein by reference in its entirety. With these crosslinking agents, one of the reactants must have a carboxyl group and the other an amine or sulfhydryl group. The crosslinking agent first reacts selectively with the carboxyl group, preferably a carboxyl group on a protein, then is split out during reaction of the "activated" carboxyl group with an amine, preferably an amine group of W in Structure I, to form an amide linkage between the protein or biological molecule and a complexing agent of this invention, thus covalently bonding the two moieties. An advantage of this approach is that crosslinking of like molecules, e.g., complexing agents with complexing agents, can be avoided, whereas the reaction of difunctional crosslinking agents is nonselective so that unwanted crosslinked molecules can be obtained.

Additional preferred protein reactive groups include semicarbazido; thiocarbazido; thiosemicarbazido; isocyanato

and isothiocyanato; vinyl sulfonylalkyloxy, the alkylene group of which preferably contains from 2 to 10 carbon atoms and is as described for R and R_1 above; vinyl sulfonylalkylpoly(oxyalkyl)oxy, the alkylene group of the sulfonylalkyl portion of which preferably contains from 2 to 10 carbon atoms and is as described for R and R_1 above, the alkylene group of the polyoxyalkyl portion preferably contains from 2 to 10 carbon atoms and is as described for R and R_1 above, such poly(oxyalkyl) portion preferably comprising a poly(oxyethylene) group or a poly(oxyethylene)-co-poly(oxypropylene) copolymer group, and the polymer contains from 2 to about 100 monomeric oxyalkylene units; amidatoalkylaxy, the alkylene group of which preferably contains from 1 to 10 carbon atoms and is as described for R and R_1 above; hydrazidoalkyloxy, the alkylene group of which preferably contains from 1 to 10 carbon atoms and is as described for R and R_1 above; azidocarbonylalkyloxy, the alkylene group of which preferably contains from 1 to 10 carbon atoms and is as described for R and R_1 above; aryloxycarbonyloxyalkyloxy, the alkylene group of which preferably contains from 2 to 10 carbon atoms and is as described for R and R_1 above, and the aryl group of which is as described for R and R_1 above; aryloxycarbonyl(polyoxyalkyl)oxy, the aryl group of which is as described for R and R_1 above, and the alkylene group of the polyoxyalkyl portion preferably contains from 2 to 10 carbon atoms and is as described for R and R_1 above, such poly(oxyalkyl) portion preferably comprising a poly(oxyethylene) group or a poly(oxyethylene)-co-poly(oxypropylene) copolymer group, and the polymer contains from 2 to about 100 monomeric oxyalkylene units; triazines such as 4,6-dichloro-2-triazinylamino, 4,6-dichloro-2-triazinyloxy, 4,6-

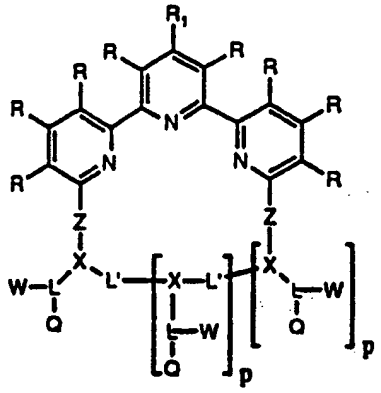
dichlorotriazinyl-2-oxy(polyalkyloxy), 4-alkoxy-6-chloro-2-triazinyloxy, and 4-alkoxy-6-chloro-2-triazinyl(polyoxyalkyl)oxy, the alkyl groups of the alkoxy portions preferably each containing from 2 to 10 carbon atoms and being as described for R and R_1 above, and the alkylene groups of the polyoxyalkyl portions preferably each containing from 2 to 10 carbon atoms and being as described for R and R_1 above, such a poly(oxyalkyl) portion preferably comprising a poly(oxyethylene) group or a poly(oxyethylene)-copoly(oxypropylene) copolymer group, in which the polymer contains from 2 to about 100 monomeric oxyalkylene units; formylalkyl, the alkyl group of which preferably contains from 1 to 10 carbon atoms and is as described for R and R_1 above; aminoalkyl, the alkyl group of which preferably contains from 1 to 10 carbon atoms and is as described for R and R_1 above; active esters, for example, succinimidoxycarbonyl; active anhydrides and mixed anhydrides; active carbonates such as arylcarbonatoaryl, alkylcarbonatoaryl, arylcarbonatoalkyl, and alkylcarbonatoalkyl, the alkyl groups of which preferably contain from 2 to 10 carbon atoms and are as described for R and R_1 above, and the aryl groups of which are preferably comprised of a six membered ring containing electron withdrawing substituents such as, for example, nitro and halogen, and optionally containing water solubilizing groups such as a sulfonate salt; sulfhydryl; sulfhydrylalkyl, the alkyl group of which preferably contains from 1 to 10 carbon atoms and is as described for R and R_1 above; thioalkylcarbonylamincalkyloxy, the alkylene group of the thioalkylcarbonyl portion preferably containing from 1 to 10 carbon atoms and being as described for R and R_1 above, and the alkylene group of the aminoalkyloxy portion preferably containing from 2 to 10 carbon atoms and being as described

for R and R_1 above; maleimidoalkylcarbonylaminoalkylaxy, the alkylene group of the maleimidoalkylcarbonyl portion preferably containing from 1 to 10 carbon atoms and being as described for R and R_1 above, and the alkylene group of the aminoalkyloxy portion preferably containing from 2 to 20 carbon atoms and being as described for R and R_1 above; azido; iodoalkylcarbonylamino, the alkylene group of which contains from 1 to 10 carbon atoms and is as described for R and R_1 above; amidatoalkylamino, the alkylene group of which contains from 1 to 10 carbon atoms and is as described for R and R1 above; and amidatoarylalkylamino, the alkylene group of which contains from 1 to 10 carbon atoms and is as described for R and R_1 above, and the aryl group of which is as described for R and R_1 above.

Especially preferred protein reactive groups include sulfhydryl, amino, isothiocyanato and arylcarbonatoalkyl.

Preferred classes of complexing agents of use in the targeting immunoreagents of

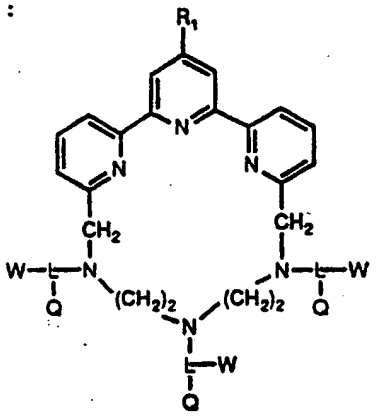
the invention include macrocyclic terpyridines having structure II:



Structure II

wherein R, R_1, Z, L, L', Q, W and p are as described above for Structure I.

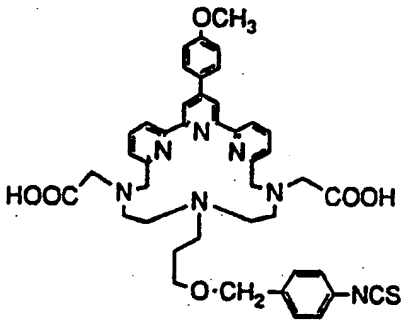
An especially preferred class of complexing agents includes macrocyclic terpyridines having structure III:

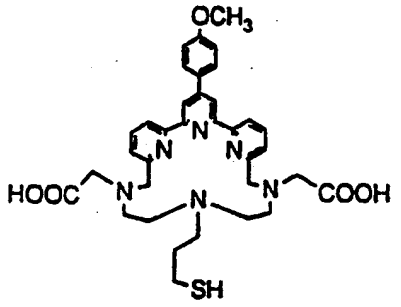


Structure III

wherein R_1, L, Q, and W are as described above for Structure I.

Specific examples of preferred complexing agents include:





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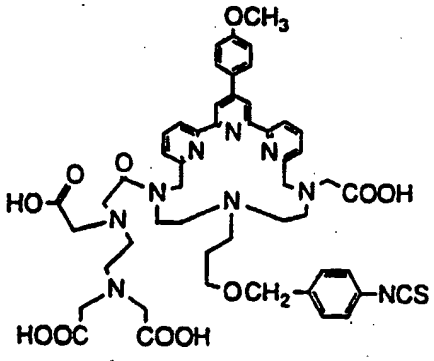
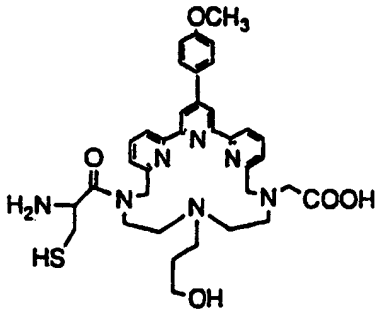
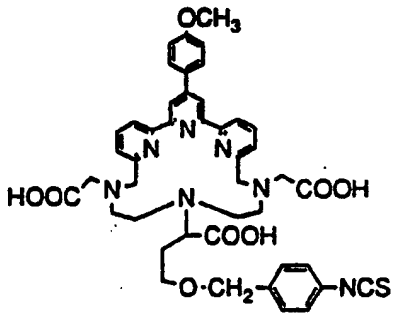
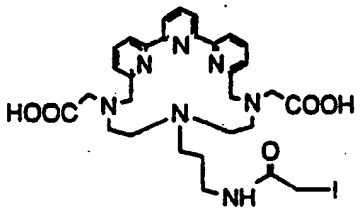
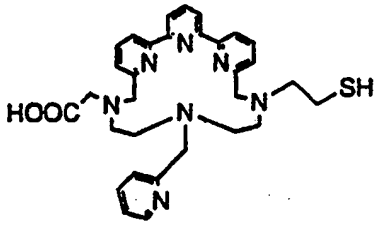
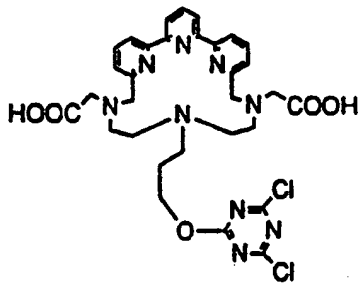






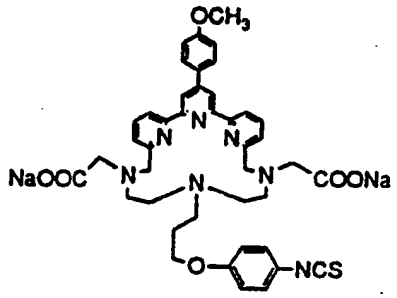




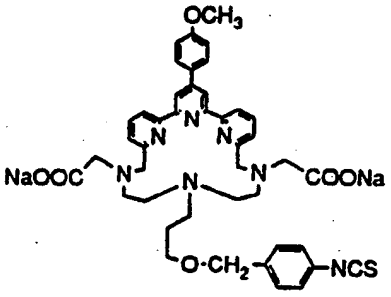


The macrocylic oligo-2,6-pyridine complexing agents can have multiple metal complexing sites, e.g., oligo-2, 6-pyridine sites and additional heteroatom sites. Suitably substituted oligo-2,6-pyridine moieties can be prepared by techniques known in the art

US Patent No. 5760191 comprises a comprehensive discussion of the preparation of the targeting immunoreagents of the invention.



39a



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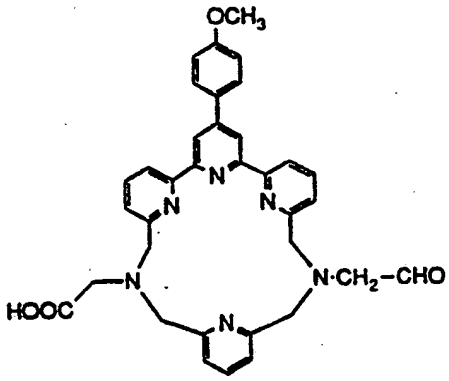

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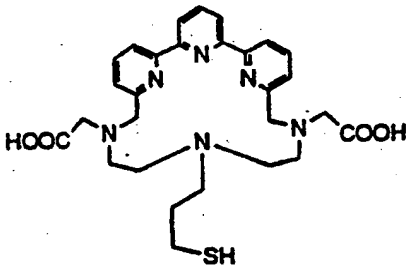

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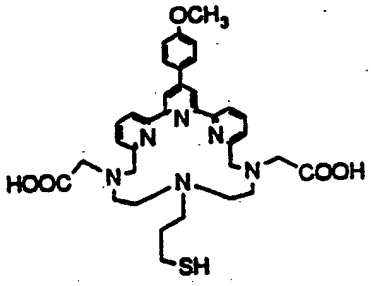
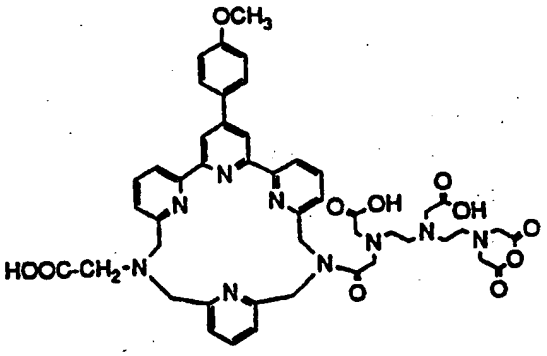




The targeting immunoreagents of the invention comprise a metal ion. The term 'metal ion' as used herein is intended to include any ion of an element other than hydrogen that has an oxidation state equal to or greater than 1 and which can bind to a complexing agent of this invention through interaction with sites of high electron density in the complexing agent such as at heteroatom sites. The interaction of the metal ion with sites of high electron density in the complexing agent can be in the form of a Lewis acid-base interaction, wherein the oxidation state of metal ion is stabilized by interaction with donated electron density from sites of high electron density of the complexing agent. A metal ion can also interact with sites of high electron density in the complexing agent to form a salt in the form of an ionic association between a positively charged metal ion such as a lanthanide ion or a yttrium ion and a negatively charged substituent on the macrocyclic complexing agent such as a carboxylate anion substituent or a phosphonate anion substituent. A metal ion can also interact with sites of high electron density in the complexing agent to form a covalent bond between the metal which has an oxidation state equal to or greater than 1 such as rhenium or technitium and a heteroatom of the macrocyclic complexing agent such as a sulfur or nitrogen or oxygen atom.

It is desirable that the metal ion be easily complexed to the chelating agent, for example, by merely exposing or mixing an aqueous solution of the chelating agent with a metal salt, preferably in an aqueous solution. Preferably such solution has a pH in the range of about 4 to about 11. The metal ion salt can be any

composition containing the metal ion. Salts with a low water solubility are useful, but preferably the salt is a water soluble salt of the metal such as, for example, a halogen or nitrate salt. More preferably such salts are selected so as not to interfere with the binding of the metal ion with the chelating agent. The chelating agent is preferably in aqueous solution at a pH of between about 5 and about 9, more preferably between about 6 and about 8.

The chelating agent can be mixed with buffer salts such as citrate, acetate, phosphate and borate to produce the optimum pH. Preferably, said buffer salts are selected so as not to interfere with the subsequent binding of the metal ion to the chelating agent. A presently preferred buffer is sodium acetate plus acetic acid in water.

In addition to ions of alkali metals such as sodium, potassium, and cesium, and to ions of alkaline earth metals such as magnesium, calcium, and barium, preferred metal ions can be selected from, but are not limited to, ions of elements of groups IIA through VIA. Preferred metals include those of atomic number 12, 13, 20, the transition elements 21 to 33, 38 to 52, 56, 72 to 84 and 88 and those of the lanthanide series (atomic number 57 to 71). Ions of yttrium and the lanthanides metals are especially preferred.

In another embodiment, the immunoreagent of this invention can comprise a fluorescent metal ion. The fluorescent metal ion can be selected from, but is not limited to, metals of atomic number 57 to 71. Ions of the following metals are preferred: La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu. Eu^{+3} is especially preferred.

Such immunoreagents can exhibit utility in time delayed fluorescence and assays which involve time delayed fluorescence such as in the detection of fluorescent metal ions such as Eu^{+3}. In such an assay the targeting fluorescent immunoreagent is irradiated with an excitation light such as, for example, a pulse of light having a maximum intensity at a wavelength of about 385 nanometers. The excitation light pulse is then stopped or blocked from further access to the metal complex, an effective time such as about 400 microseconds is allowed to elapse, and emission of light is then detected and measured with a detector capable of determining intensity of light as a function of wavelength. In particular, it is desirable that the wavelength of the emitted light be different from the wavelength of the excitation light, and that the effective time delay be about 400 microseconds or longer so that no interference from ambient fluorescent emitters interferes with the assay. A preferred composition for this type of assay comprises macrocycle (39a) chelated to a Eu^{+3} ion.

In another embodiment, the metal ion of this invention can comprise a paramagnetic metal ion which is suitable for use in nuclear magnetic resonance applications which include diagnostic imaging using MRI techniques. The paramagnetic element can be selected from elements of atomic number 21 to 29, 43, 44 and 57 to 71. The following elements are preferred: Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. Mn, Gd, and Dy are especially preferred.

In another embodiment, the metal ion of this invention can comprise a radionuclide. The radionuclide can be selected, for example, from radioisotopes of Sc, Fe, Pb, Ga, Y, Bi, Lu, Mn, Cu, Cr, Zn, Ge, Mo, Tc, Ru, In, Sn, Sm, Sr, Eu, Dy, Sb, W, Re, Po, Ta and Tl. Preferred radionuclides include ^{44}Sc, ^{64}Cu, ^{67}Cu, ^{111}In, ^{212}Pb, ^{68}Ga, ^{90}Y, ^{87}Y, ^{153}Sm, ^{212}Bi, ^{99m}Tc, ^{186}Re and ^{188}Re. Of these, especially preferred is ^{90}Y.

In some applications, a metal chelate of a mixture of metal ions such as sodium ions and yttrium ions is useful. For example, a solution of a metal complexing (or chelating) agent of this invention such as compound (39a) in a sodium acetate buffer can be treated with a less than stoichiometric quantity of a radionuclide such as ^{90}Y, and after a sufficient time during which chelation of substantially all of the radionuclide occurs, the subsequent mixture containing ^{90}Y bound to metal chelate plus the sodium salt of non-^{90}Y-containing metal chelate can be useful without further separation of the individual components, for example, in radioscintigraphic analysis of proteins separated by electrophoresis. In bulk solution, the metal chelate of this aspect of this invention preferably contains a ratio of metal radionuclide ion to chelating agent that is effective in such applications. In preferred embodiments, the mole ratio of metal ion per chelating agent is from about 1:1000 to about 1:1.

The targeting immunoreagent of this invention includes an immunoreactive group bonded, by a linking group that comprises the residue of a protein reactive group, to the macrocyclic complexing agent. The targeting immunoreagent thus comprises a conjugate of a complexing agent having the structure I above and the immunoreactive group. The complexing agent and the metal can be complexed either before or after the complexing agent is attached to the immunoreactive group.

The immunoreactive group is

an ST receptor binding moiety, such as ST enterotoxins or analogues thereof which bind to the ST receptors which are found only on the apical brush border membranes of the cells lining the intestinal tract of placental mammals.

A variety of bacteria, such as Eschericia coli, Vibrio cholerae, Citrobacter freundii and Yersinia enterocolitica, which may infect the mammal gut produce homologous peptide toxins which bind to ST receptors and trigger a cascade of biochemical processes eventually leading to fluid secretion into the intestinal lumen and hence diarrhoea. These ST enterotoxins are a major cause of infectious diarrhoeal disease in developing countries, the fourth leading cause of mortality and morbidity in the pediatric population worldwide. These enterotoxins typically contain 18 or 19 amino acid residues, are stable

to proteases and maintain their bioactivity even after incubation at 100°C for 15 minutes. Examples of such heat stable ST enterotoxins are listed in the table below:

Table 1

Source	Structure
E.Coli STa	Asn-Thr-Phe-Tyr-Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr
E.Coli STh	Asn-Ser-Ser-Asn-Tyr-Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr
Yersinia ST	Ser-Ser-Asp-Trp-Asp-Tyr-Cys-Cys-Asp-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr
Vibrio ST	Ile-Asp-Cys-Cys-Glu-Ile-Cys-Cys-Asn-Pro-Ala-Cys-Phe-Gly-Cys-Leu-Asn
Guanylin	Pro-Gly-Thr-Cys-Glu-Ile-Cys-Ala-Tyr-Ala-Ala-Cys-Thr-Gly-Cys
Uroguanylin	Asn-Asp-Asp-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu

The naturally occurring ST enterotoxins have a relatively complicated secondary structure, due for example to the presence of multiple disulphide bridges. Thus E.Coli STa has disulphide bridges between the Cys residues at positions 5 and 10, 6 and 14 and 9 and 17.

Such cell receptor binding oligopeptides and analogues thereof are of interest both for therapeutic and diagnostic purposes.

Thus for example an oligopeptide capable of binding to a cell surface receptor may be coupled to the complexing agents of the invention and serve as a biological vector to deliver that moiety to sites possessing such cell surface receptors. Thus radiopharmaceuticals may be caused to accumulate at body sites having the target receptors and so

allow such sites to be detected and if desired mapped. Where the receptor occurs solely or predominantly on undesired cells, e.g. tumour cells, a cytotoxic dose of radiation can likewise be delivered to the site of concern using radiation emitting vector-bound radionuclides.

In the case of the heat stable ST enterotoxins mentioned above, the ST receptors occur naturally only in the intestinal lumen and are found elsewhere in the body only as a result of metastases of colon cancers. Parenteral administration of a radionuclide-tagged ST oligopeptide can be used to detect and treat such metastases (see US-A-5518888 and WO95/11694).

In Waldman (US-A-5518888) a method of imaging metastasized colorectal cancer cells is disclosed using a ST receptor binding moiety. This citation is herein incorporated by reference.

If desired, the immunoreactive group can be modified or chemically altered to provide reactive groups for attaching to the chelating agent by techniques known to those skilled in the art. Such techniques include the use of linking moieties and chemical modification such as described in WO-A-89/02931 and WO-A-89/2932, which are directed to modification of oligonucleotides, and U.S. Patent No. 4,719,182.

Two highly preferred uses for the targeting immunoreagent compositions of this invention are for the diagnostic imaging of tumors and the radiological treatment of tumors.

The immunoreactive material contains a reactive site that comprises a reactive group that can react or combine with the protein reactive group on the chelating agent as defined in Structure 1 to form a linking group between the immunoreactive material and the chelating agent. Suitable reactive sites on the immunoreactive material include amine sites of lysine; terminal peptide amines; carboxylic acid sites, such as are available in aspartic acid and glutamic acid residues; sulfhydryl sites, such as in cysteine residues; carbohydrate sites and oxidized carbohydrate sites; activated carbonhydrogen and carbon-carbon bonds which can react through insertion via free radical reaction or nitrene or carbene reaction of an activated residue; sites of oxidation including, for example, a vicinal diol site of a carbohydrate moiety and a serine alcohol, each of which can be oxided to an aldehyde; sites of reduction, for example a disulfide linkage which can be reduced to form a sulfhydryl group; aromatic sites such as the hydroxyaromatic group of tyrosine; and hydroxyl sites such as the phenolic hydroxyl group of tyrosine, the hydroxyl group of serine, and the hydroxyl group of a carbohydrate moiety.

In one aspect, the phrase "residue of a linking group" as used herein refers to a moiety that remains, results, or is derived from the reaction of a protein reactive group with a reactive site on a protein. The phrase "protein reactive group" as used herein refers to any group which can react with functional groups typically found on proteins. However, it is specifically contemplated that such protein reactive groups can also react with functional groups typically found on relevant nonprotein molecules.

Preferred linking groups are derived from protein reactive groups selected from but not limited to:

(1) a group that will react directly with amine, alcohol, or sulfhydryl groups on the immunoreactive protein or biological molecule containing the reactive group, for example, active halogen containing groups including, for example, chloromethylphenyl groups and chloroacetyl [$ClCH_2C(=O)-$] groups, activated 2-(leaving group substituted)-ethylsulfonyl and ethylcarbonyl groups such as 2-chloroethylsulfonyl and 2-chloroethylcarbonyl; vinylsulfonyl; vinylcarbonyl; epoxy; isocyanato; isothiocyanato; aldehyde; aziridine; succinimidoxycarbonyl; activated acyl groups such as carboxylic acid halides; mixed anhydrides and the like; and other groups known to be useful in conventional photographic gelatin hardening agents;

(2) a group that can react readily with modified proteins or biological molecules containing the immunoreactive group, i.e., proteins or biological molecules containing the immunoreactive group modified to-contain reactive groups such as those mentioned in (1) above, for example, by oxidation of the protein to an aldehyde or a carboxylic acid, in which case the "linking group" can be derived from protein reactive groups selected from amino, alkylamino, arylamino, hydrazino, alkylhydrazino, arylhydrazino, carbazido, semicarbazido, thiocarbazido, thiosemicarbazido, sulfhydryl, sulfhydrylalkyl, sulfhydrylaryl, hydroxy, carboxy, carboxyalkyl and carboxyaryl, the alkyl portions of which linking groups contain from 1 to about 20 carbon atoms and the aryl portions of which linking groups contain from about 6 to about 20 carbon atoms; and

(3) a group that can be linked to the protein or biological molecule containing the immunoreactive group, or

to the modified protein as noted in (1) and (2) above by use of a crosslinking agent. The residues of certain useful crosslinking agents, such as, for example, homobifunctional and heterobifunctional gelatin hardeners, bisepoxides, and bisisocyanates can become a part of a linking group during the crosslinking reaction. Other useful crosslinking agents, however, can facilitate the crosslinking, for example, as consumable catalysts, and are not present in the final conjugate. Examples of such crosslinking agents are carbodiimide and carbamoylonium crosslinking agents as disclosed in U.S. Patent No. 4,421,847 and the ethers of U.S. Patent No. 4,877,724. With these crosslinking agents, one of the reactants such as the immunoreactive group must have a carboxyl group and the other, such as the complexing agent, must have a reactive amine, alcohol, or sulfhydryl group. In amide bond formation, the crosslinking agent first reacts selectively with the carboxyl group, then is split out during reaction of the thus "activated" carboxyl group with an amine to form an amide linkage between the immunoreactive group and the chelating agent and thus covalently bonding the two moieties. An advantage of this approach is that crosslinking of like molecules is avoided, whereas the reaction of, for example, homo-bifunctional crosslinking agents is nonselective and unwanted crosslinked molecules are obtained.

Useful linking groups are derived from various heterobifunctional cross-linking reagents such as those listed in the Pierce Chemical Company Immunotechnology Catalog - Protein Modification Section, (1991 and 1992). Useful non-limiting examples of such reagents include: Sulfo-SMCC, i.e., Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate; Sulfo-SIAB, i.e., Sulfosuccinimidyl (4-iodoacetyl)aminobenzoate; Sulfo-SMPB,

i.e., Sulfosuccinimidyl 4-(pmaleimidophenyl)butyrate; 2-IT, i.e., 2-Iminothiolane; and SATA, i.e., N-Succinimidyl S-acetylthioacetate.

In addition to those described above, the linking groups, in whole or in part, can also be comprised of and derived from complementary sequences of nucleotides and residues of nucleotides, both naturally occurring and modified, preferably non-self-associating oligonucleotide sequences. Particularly useful, non-limiting examples of reagents for incorporation of modified nucleotide moieties containing reactive functional groups, such as amine and sulfhydryl groups, into an oligonucleotide sequence are commercially available from, for example, Clontech Laboratories Inc. (Palo Alto, California) and include Uni-Link AminoModifier (Catalog # 5190), Biotin-ON phosphoramidite (Catalog # 5191), N-MNT-C6-AminoModifier (Catalog # 5202), AminoModifier II (Catalog # 5203), DMT-C6-3'Amine-ON (Catalog # 5222), C6-ThiolModifier (Catalog # 5211), and the like.

In one aspect, linking groups of this invention are derived from the reaction of a reactive functional group such as an amine or sulfhydryl group as are available in the above Clontech reagents, one or more of which has been incorporated into an oligonucleotide sequence, with, for example, one or more of the previously described protein reactive groups such as heterobifunctional protein reactive groups, one or more of which has been incorporated into, for example, an immunoreactive material as described above.

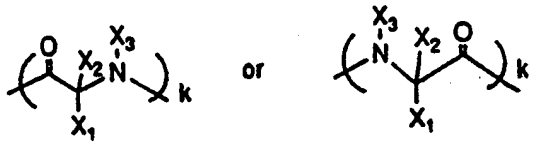
Respectively complementary individual oligonucleotide sequences are attached to the two components of the conjugate, one sequence to the immunoreactive material and the complementary oligonucleotide sequence to the chelating agent. The hybrid formed between the two

complementary oligonucleotide sequences then comprises the linking group between the immunoreactive material and the chelating agent.

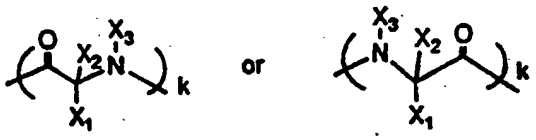
If desired, two or more copies of the same oligonucleotide sequence can be linked, for example, in tandem to one immunoreactive material, and a complementary oligonucleotide sequence comprised of multiple chelating agents can be added. The multiple hybrids formed between the two complementary oligonucleotide sequences then comprises the linking group between the immunoreactive group and multiple chelating agents.

Preferred linking groups also include nitrogen atoms in groups such as amino, imido, nitrilo and imino groups; alkylene, preferably containing from 1 to 18 carbon atoms such as methylene, ethylene, propylene, butylene and hexylene, such alkylene optionally being interrupted by 1 or more heteroatoms such as oxygen, nitrogen and sulfur or heteroatom-containing groups; carbonyl; sulfonyl; sulfinyl; ether; thioether; ester, i.e., carbonyloxy and oxycarbonyl; thioester, i.e., carbonylthio, thiocarbonyl, thiocarbonyloxy, and oxythiocarboxy; amide, i.e., iminocarbonyl and carbonylimino; thioamide, i.e., iminothiocarbonyl and thiocarbonylimino; thio; dithio; phosphate; phosphonate; urelene; thiourelene; urethane,

i.e., iminocarbonylexy,and oxycarbonylimino; an amino acid linkage, i.e., a



group wherein k=1 and X_1, X_2, X_3 are independently selected from H; alkyl, containing from 1 to 18, preferably 1 to 6 carbon atoms, such as methyl, ethyl and propyl, such alkyl optionally being interrupted by 1 or more heteroatoms such as oxygen, nitrogen and sulfur; substituted or unsubstituted aryl, containing from 6 to 18, preferably 6 to 10 carbon atoms such as phenyl, hydroxyiodophenyl, hydroxyphenyl, fluorophenyl and naphthyl; aralkyl, preferably containing from 7 to 12 carbon atoms, such as benzyl; heterocyclyl, preferably containing from 5 to 7 nuclear carbon and one or more heteroatoms such as S, N, P or O, examples of preferred heterocyclyl groups being pyridyl, quinolyl, imidazolyl and thienyl; heterocyclylalkyl, the heterocyclyl and alkyl portions of which preferably are as described above; or a peptide linkage, i.e., a



group wherein k>1 and each X independently represents a group as described for X_1, X_2, X_3 above. Two or more linking groups can be used, such as, for example, alkyleneimino and iminoalkylene. It is contemplated that other linking groups

may be suitable for use herein, such as linking groups commonly used in protein heterobifunctional and homobifunctional conjugation and crosslinking chemistry as described above. Especially preferred linking groups include amino groups which, when linked to the residue of a chelating agent via an isothiocyanate group on the chelating agent, form thiourea groups.

The linking groups can contain various substituents which do not interfere with the coupling reaction between the chelating agent of this invention and the immunoreactive group. The linking groups can also contain substituents which can otherwise interfere with such reaction, but which during the coupling reaction, are prevented from so doing with suitable protecting groups commonly known in the art and which substituents are regenerated after the coupling reaction by suitable deprotection. The linking groups can also contain substituents that are introduced after the coupling reaction. For example, the linking group can be substituted with substituents such as halogen, such as F, Cl, Br or I; an ester group; an amide group; alkyl, preferably containing from 1 to about 18, more preferably, 1 to 4 carbon atoms such as methyl, ethyl, propyl, i-propyl, butyl, and the like; substituted or unsubstituted aryl, preferably containing from 6 to about 20, more preferably 6 to 10 carbon atoms, such as phenyl, naphthyl, hydroxyphenyl, iodophenyl, hydroxyiodophenyl, fluorophenyl and methoxyphenyl; substituted or unsubstituted aralkyl, preferably containing from 7 to about 12 carbon atoms, such as benzyl and phenylethyl; alkoxy, the alkyl portion of which preferably contains from 1 to 18 carbon atoms as described for alkyl above; alkoxyaralkyl, such as ethoxybenzyl; substituted or unsubstituted heterocyclyl, preferably containing from 5 to 7 nuclear carbon and

heteroatoms such as S, N, P or O, examples of preferred heterocyclyl groups being pyridyl, quinolyl, imidazolyl and thienyl; a carboxyl group; or a carboxyalkyl group, the alkyl portion of which preferably contains from 1 to 8 carbon atoms.

The products of the reaction of any of these protein reactive group containing chelating agents with immunoreactive materials, preferably with proteins, can be purified by conventional techniques such as diafiltration, HPLC, electrophoresis, and the like. The immunoreactive materials may be subsequently modified with agents such as PEG (polyethylene glycol) reagents as is well known in the art to impart reduced immunogenicity to the modified proteins.

Techniques for performing the covalent binding of the immunoreactive group to the metal complexing agents are known in the art and include simply mixing the materials together, preferably in aqueous solution in the presence of a buffer salt such as sodium borate or sodium phophate, or sodium acetate at a pH of about 4 to about 11, preferably from about 7 to about 10.

The ratio of the complexing agent to the immunoreactive group can vary widely from about 0.5:1 to 10:1 or more. In some embodiments, the mole ratio of complexing agent to immunoreactive groups is from about 1:1 to about 6:1. In some uses of the immunoconjugates of this invention, the bulk ratio of the chelating agent to the immunoreactive group can be an apparent fraction because the immunoconjugate can be used in the presence of unmodified immunoreactive material. The immunoreagents of this invention can contain a wide range of ratios of metal ion to complexing agent. In preferred embodiments, the mole ratio of metal ion to complexing agent is from about 1:1000 to about 1:1. The ratio of the complexing agent to the immunoreactive group can vary widely from about 0.5:1 to 10:1 or more. In some embodiments, the mole ratio of complexing agent to immunoreactive groups is from about 1:1 to about 6:1.

The targeting immunoreagent of this invention comprising a radioisotope of a metal ion such as $^{90}Y^{+3}$ (as a non-limiting example) can be used for the therapeutic treatment of tumors, particularly if the immunoreagent is a tumor antigen specific antibody or a fragment of such antibody. In therapeutic applications, the targeting immunoreagent of this invention preferably contains a ratio of metal radionuclide ion to chelating agent that is effective in such therapeutic applications. In preferred embodiments, the mole ratio of metal ion per chelating agent is from about 1:100 to about 1:1.

The targeting immunoreagent of this invention comprising a radioisotope of a metal ion such as $^{111}In^{+3}$ or $^{187}Y^{+3}$ (as non-limiting examples) can be used for the diagnostic imaging of tumors in cancer patients, particularly if the immunoreagent is a tumor antigen specific antibody or a fragment of such antibody. In diagnostic imaging, applications, the targeting immunoreagent of this invention preferably contains a ratio of metal radionuclide ion to chelating agent that is effective in such diagnostic imaging applications. In preferred embodiments, the mole ratio of metal ion per chelating agent is from about 1:10,000 to about 1:1.

In another embodiment of this invention, a targeting immunoreagent as described above comprising at least two metal ions in combination with one another in the same formulation is specifically contemplated. For example,

the use of a therapeutically effective dose of a radionuclide such as $^{90}Y^{+3}$ together with a diagnostic imaging effective dose of a paramagnetic ion such as Gd^{+3}, the ratio of the molar concentration of the diagnostic imaging effective ion to the molar concentration of the radionuclide ion being typically greater than one, in a pharmaceutically effective formulation of said targeting immunoreagent, permits the simultaneous magnetic resonance imaging of at least a portion of the tissue of a host patient during therapeutic treatment of said patient.

In another embodiment of this invention, the use of radioisotopes of iodine is specifically contemplated. For example, if the targeting immunoreagent comprises a substituent that can be chemically substituted by iodine in a covalent bond forming reaction, such as, for example, a substituent containing hydroxyphenyl functionality, such a substituent can be labeled by methods well known in the art with a radioisotope of iodine. The thus covalently linked radioactive iodine species can be used in therapeutic and diagnostic imaging applications as described herein.

In a preferred embodiment, an effective dose of a targeting radioactive immunoreagent as described above in a pharmaceutically acceptable medium is prepared by exposing a composition comprising a residue of an immunoreactive group as described above and a residue of a chelating agent having Structure I as described above linked to the immunoreactive group by a linking group as described above to a composition containing a radioactive metal ion as described above such that the molar amount of said radioactive metal ion is less than the molar amount of the chelating group comprising the targeting immunoreagent in said composition, the duration of the exposure lasting an effective time to permit uptake of

said radioactive metal ion into said targeting immunoreagent.

In a preferred embodiment, an effective dose of a targeting immunoreagent as described above in a pharmaceutically acceptable medium is administered to a patient and said targeting immunoreagent is allowed to accumulate at the target site such as at a tumor site in said patient.

In a preferred embodiment, a therapeutically effective dose of a targeting radioactive immunoreagent as described above in a pharmaceutically acceptable medium is administered to a patient or to a tissue from a patient and said targeting radioactive immunoreagent is allowed to accumulate at the target site such as at a tumor site in said patient.

The present invention also comprises one or more targeting immunoreagents as described above formulated into compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants or vehicles which are collectively referred to herein as carriers, for parenteral injection, for oral administration in solid or liquid form, for rectal or topical administration, or the like.

The compositions can be administered to humans and animals either orally, rectally, parenterally (intravenously, by intramuscularly or subcutaneously), intracisternally, intravaginally, intraperitoneally, intravesically, locally (powders, ointments or drops), or as a buccal or nasal spray. It is specifically contemplated that a targeting paramagnetic immunoreagent as described above and targeting radioactive immunoreagent as described above can be administered by the same route. It is also contemplated that a paramagnetic immunoreagent as described above can be administered by a route different from that of a targeting radioactive immunoreagent as described above.

The formulation of compositions suitable for oral, rectal, parenteral, intercisternal, intravaginal, intraperitoneal, intravesical, topical, buccal or nasal is well known in the art and is comprehensively described in US Patent No. 5760191.

Actual dosage levels of active ingredients in the compositions of the present invention may be varied so as to obtain an amount of active ingredient that is effective for imaging or to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired imaging or therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

The total daily dose of the compounds of this invention administered to a host in single or divided dose may be in amounts, for example, of from about 1 nanomol to about 5 micromols per kilogram of body weight. Dosage unit compositions may contain such amounts or such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

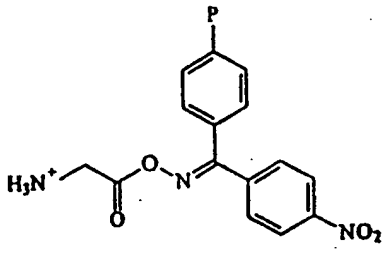
After administration of a composition of the present invention, the subject mammal is maintained for a time period sufficient for the administered composition to be distributed throughout the subject and enter the tissues of the mammal. A sufficient time period is generally from about 1 hour to about 2 weeks or more and, preferably from about 2 hours to about 1 week.

The following examples further illustrate the invention

Example 1 - Preparation of an ST-TMT conjugate

Part A Preparation of Gly-Oxime Resin

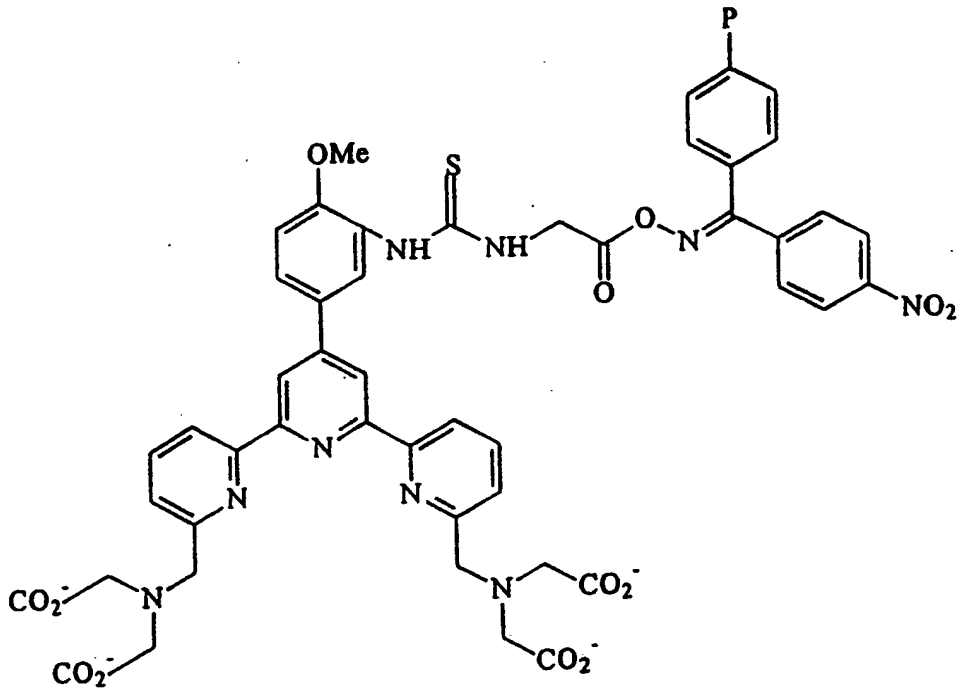
Five grams of p-nitrobenzophenone oxime resin was mixed with 100 ml $CHCl_3$ containing 15 mmoles Boc-Gly-N-hydroxybenzotriazole (HOBT) ester (the Boc-Gly-HOBT ester was made by mixing HOBT and diisopropyl-carbodiimide (1:1) with Boc-Gly, and added to $CHCl_3$ immediately prior to mixing with the oxime resin). The mixture was stirred overnight, washed three times each with dimethylformamide (DMF) and $CHCl_3$, and deprotected for 30 minutes with 40% TFA/$CHCl_3$ to form the title compound. The product (1) was then washed three times each with dimethylformamide (DMF) and $CHCl_3$ and air dried under vacuum.



P=polystyrene/divinylbenzene

Part B Preparation of TMT-Gly-Oxime Resin

10 mg of Gly-Oxime Resin prepared as above was combined with 15 micromoles of TMT-NCS prepared as in WO 92/08494. Two hundred microliters of 1% diisopropyl-ethylamine (DIEA) in DMF was added, and the reaction stirred at 37°C for five hours. The reaction was washed three times each in DMF, dichloromethane, and MeOH, then air dried to form the title compound.



Part C Cleavage with ST to form TMT-ST

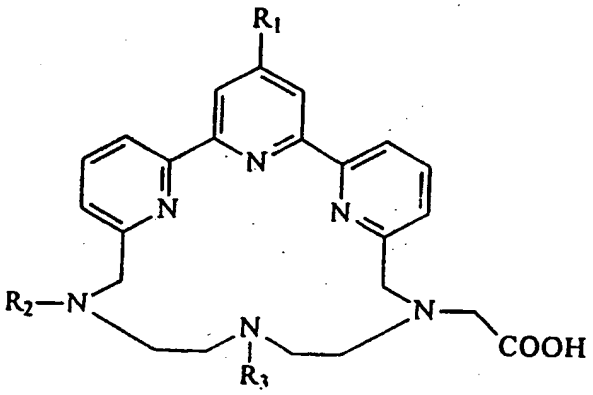
One milligram of ST powder (92% peptide, w/w) was added to 5 mg of TMT-Gly-Oxime Resin prepared as above. 200 microliters of DMF containing 0.5 micromoles DIEA (1.0 equivalent relative to ST) was then added and the mixture stirred at room temperature overnight to form title compound.

Example 2

A solution of compound A below (6 mmoles) in an appropriate solvent such as DMF containing 1% (v/v) diisopropylethylamine is added to a solution of compound B below (1 mmole) in a similar miscible solvent such as DMF, water,

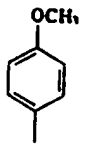
N-methylpyrrolidone or dimethylsulfoxide and stirred at 20-40°C for 1-24 hours. The resulting conjugate is readily purified by chromatographic methods and verified by chromatographic and spectrographic methods.

Compound A:

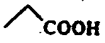


wherein:

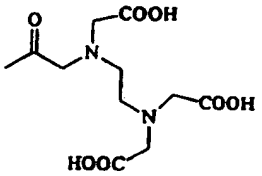
R_1 =



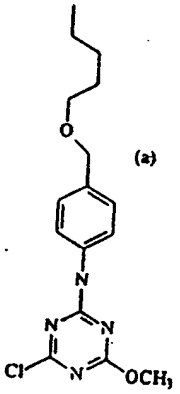
R_2 =



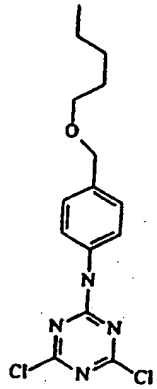
or



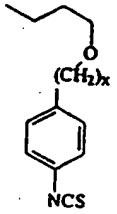
R_3 =



(a)

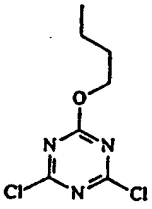


(b)

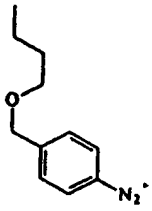


x = 0 or 1

(c)



(d)



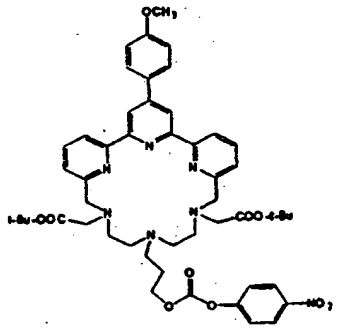
(e)

For compounds in which R_3 is structure (b) or (d), an excess of the ST enterotoxin is used relative to the ligand. For compounds in which R_3 is structure (e) the ST enterotoxin has only one tyrosine positioned at residues 1-4.

Compound B:

Single primary amine ($R-NH_2$) in an ST enterotoxin or a derivative, fragment or analog thereof.

Example 3 3,9-Bis(t-butoxycarbonymethyl)-18-(4-methoxyphenyl)-6-[3-(4-nitrophenyl)-oxycarbonyl-oxypropyl]-3,6,9,25,26,27-hexaazatetracyclo-[19.3.1.$1^{11,15}$.$1^{16,20}$]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene.



To a magnetically stirred solution of 100 mg of the compound prepared in Example 39 of US Patent No. 5760191 and one equivalent of 2, 6-lutidine in 20 mL of dry methylene chloride cooled in an ice bath under dry argon is added a solution of one equivalent of 4-nitrophenylchloroformate (CAS Registry No. 7693-46-1; Senn Chemicals AG) in 10 mL of methylene chloride. After 2 hours, the reaction mixture is extracted twice with equal volumes of ice water. The organic layer is separated and dried in a dry, ice-cooled flask over sodium sulfate, filtered, and concentrated to near dryness below 10°C. The product is isolated by trituration with a mixture of cold ethyl acetate in hexane.

Example 4. Preparation of Escherichia Coli Heat-Stable Enterotoxin (STa) conjugate with 3,9-bis(carboxymethyl)-18-(4-methoxyphenyl)-6-[3-(4-nitrophenyl)oxycarbonyloxypropyl]-3,6,9,25,26,27-hexaazatetracyclo[19.3.1.1^{11,15}.1^{16,20}]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene (STa-chelate$_{58}$).

A 10 mL amber Reacti-Vial™ with magnetic stirrer (Pierce Life Science) is pretreated for 24 hours with a saturated solution of KOH in ethanol, then with an aqueous solution of DTPA, then washed with water, 1 N hydrochloric acid, 1 N ammonium hydroxide, and then again with water. The vial is then silanized by treatment with trimethylchlorosilane and then dried at 110°C. A magnetically stirred solution of 2 mg of Escherichia Coli heat-stable enterotoxin (STa; Sigma Biochemicals, product E5763) and one equivalent of dry diisopropylethylamine (Aldrich) in 5 mL of anhydrous dimethylformamide (freshly distilled from calcium hydride) in the Reacti-Vial™ is treated at ambient temperature under argon with 2 mg of the compound prepared in Example 3. The reaction is followed by size exclusion HPLC. When the reaction is substantially complete, the product is diluted with excess trifluoroacetic acid in water to hydrolyze the t-butyl esters, and the product can be isolated by preparative HPLC.

Example 5. General method for radiolabeling of a chelating agent of this invention as a conjugate of STa with radionuclide ion illustrated using the STa-chelate$_{58}$ and ^{90}Y.

A volume of radioactive yttrium chloride ($^{90}YCl_3$ in 0.04 M hydrochloric acid at a specific activity of >500 Ci/mg; Amersham-Mediphysics) is neutralized using two volumes of 0.5 M sodium acetate, pH 6.0. The neutralized ^{90}Y (1.0 mCi) is added to 1.0 mL of STa-chelate$_{58}$ (1 mg/mL) in 50 mM sodium acetate buffer containing 150 mM sodium chloride at

pH 5.6. The labeling is allowed to proceed for one hour and then the reaction mixture is loaded onto a PD-10 chromatography column which has been prewashed and equilibrated in a buffer containing 50 mM sodium phosphate with 150 mM sodium chloride pH 7.4 (PBS). The sample is eluted from the column with PBS. Fractions of radiolabeled Sta-chelate$_{58}$ are collected, assayed for radioactivity, and pooled. The labelling efficiency is determined by removing 0.5 mL of the sample and spotting it on to a Gelman ITLC-SG strip. The strip is developed in a glass beaker containing 0.1 M sodium citrate, pH 6.0, for a few minutes until the solvent front has reached three quarters of the way to the top of the paper. The strip is inserted into a System 200 Imaging scanner (Bioscan) which has been optimized for ^{90}Y. In this system, free ^{90}Y migrates at the solvent front while the Sta-chelate$_{58}$-^{90}Y does not.

Example 6a. Radiolabeling of the conjugate of STa-chelate$_{58}$ with ^{111}In.

The method of Example 5 is repeated using $^{111}InCl_3$ in 0.04 M hydrochloric acid (Amersham-Mediphysics) in place of $^{90}YCl_3$.

Example 6b. Labeling of STa-chelate$_{58}$ with $EuCl_3$.

The method of Example 55 of US Patent No. 5760191 is repeated using STa- chelate$_{58}$ conjugate. Progress of the reaction is followed spectroscopically.

Example 7. Preparation of Escherichia Coli Heat-Stable Enterotoxin (STa) conjugate with disodium 3,9-bis(carboxymethyl)-18-(4-methoxyphenyl)-6-[3-(4-isothiocyanatophenylmethyl)-oxypropyl]-3,6,9,25,26,27-hexaazatetracyclo[19.3.1.1^{11,15}.1^{16,20}]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene (Sta-chelate$_{62}$).

In a 10 ml Reacti-Vial silanized as in Example 58, a solution of 1 mg of Escherichia Coli heat-stable enterotoxin (STa) in 5 mL of anhydrous DMF is treated with 2 mg of the compound prepared in Example 43 of US Patent No 5760191 and one equivalent of diisopropylethylamine. The reaction mixture is stirred at 40°C for 24 hours. The conjugate (Sta-chelate$_{62}$) can be isolated by preparative HPLC.

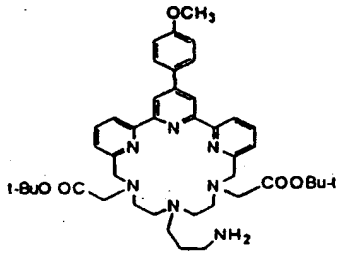
Example 8 (a). Preparation of Escherichia Coli Heat-Stable Enterotoxin (STa) conjugate with ω-amino-α-carboxylpoly(ethylene glycol)$_{3,400}$ (H_2N-PEG$_{3,400}$-CO-STa).

A solution of 1 mg of Escherichia Coli heat-stable enterotoxin (STa) and one equivalent of diisopropylethylamine in 5 mL of anhydrous DMF is treated with 2 mg of t-Boc-NH-PEG$_{3,400}$-CO2-NHS (Shearwater Chemicals; item number t-Boc-NHS, a 3,400 Dalton poly(ethylene oxide) terminated by w-t-butoxycarbonylamine and a-N-hydroxysuccinimidyl carboxylic ester groups). The reaction mixture is stirred at 40°C. Progress of the reaction is followed by size exclusion HPLC. When the reaction is complete, the t-Boc protecting group is removed with trifluoroacetic acid, and the product, H_2N-PEG$_{3,400}$-CO-STa, is isolated by preparative SE-HPLC.

Example 8 (b). Preparation of an addition product of H_2N-$PEG_{3,400}$-CO-STa with disodium 3,9-bis(carboxymethyl)-18-(4-methoxyphenyl)-6-[3-(4-isothiocyanato-phenylmethyl)-oxypropyl]-3,6,9,25,26,27-hexaazatetracyclo[19.3.1.$1^{11,15}$.$1^{16,20}$]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene (Sta-PEG-$chelate_{63}$).

In a 10 ml Reacti-Vial silanized as in Example 4, a solution of 1 mg of H_2N-$PEG_{3,400}$-CO-STa prepared in Example 65(a) and one equivalent of diisopropylethylamine in 6 mL of DMF is treated with 1 mg of the compound prepared in Example 43. of US Patent No 5760191
The reaction mixture is stirred at 40°C. The extent of reaction was followed by SE-HPLC. The conjugate (Sta-PEG-$chelate_{63}$) can be isolated by preparative SE-HPLC.

Example 9. 3,9-Bis(t-butoxy-carbonylmethyl)-18-(4-methoxy-phenyl)-6-(3-aminopropyl)-3,6,9,25,26,27-hexaazatetracyclo[19.3.1.$1^{11,15}$.$1^{16,20}$]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene.



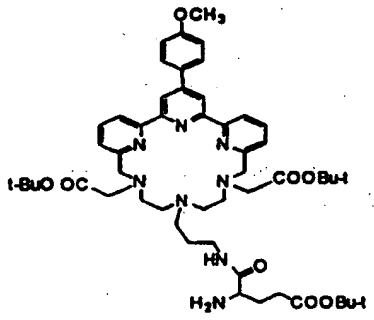
A solution of one part by weight of the compound prepared in Example 39 of US Patent No. 5760191
and one part by weight of 2,6-lutidine in 20 parts by weight of dry methylene chloride cooled in an ice bath under dry argon is treated with one equivalent of p-toluenesulfonyl chloride. After 3 hours at ice temperature, the reaction mixture is treated with excess methanolic ammonium and allowed to warm to room temperature. The solvent is evaporated and the residual material is

purified by preparative HPLC and crystallization from methanol.

Example 10. Preparation of Escherichia Coli Heat-Stable Enterotoxin (STa) conjugate with 3,9-bis(carboxymethyl)-18-(4-methoxyphenyl)-6-(3-aminopropyl)-3,6,9,25,26,27-hexaazatetracyclo-[19.3.1.$1^{11,15}$.$1^{16,20}$]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene, (STa-chelate$_{65}$).

In a 10 ml Reacti-Vial silanized as in Example 2, a solution of 1 part of Escherichia Coli heat-stable enterotoxin (STa) and 1 part of the compound prepared in Example 9 in 5 parts of anhydrous DMF is treated with 1 part of 1,1'-carbonyldiimidazole. The symmetrical products of the reaction are separated by preparative HPLC from the desired unsymmetrical conjugate which is treated with trifluoroacetic acid to remove the t-butyl groups. The desired product, STa-chelate$_{65}$, can be isolated by preparative HPLC.

Example 11. 3,9-Bis(t-butoxy-carbonylmethyl)-18-(4-methoxy-phenyl)-6-(3-aminopropyl)-3,6,9,25,26,27-hexaazatetracyclo-[19.3.1.$1^{11,15}$.$1^{16,20}$]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene-γ-t-butoxyglutamic acid amide.



A solution containing one part of the compound prepared in Example 9 and one part of 9-fluorenyl-methoxycarbonyl-L-glutamic acid-γ-t-butyl ester (Peptides International, Inc.; product Fmoc-Glu(OtBu)) in 10 parts of 50:50 DMF acetonitrile is treated with one part of benzotriazol-1-yl-oly-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and two parts of triethylamine to produce the Fmoc blocked glutamic acid amide. Progress of the reaction can be followed by HPLC. The Fmoc group on the product can be removed by the addition of a solution of piperidine in DMF, and the desired material can be isolated by preparative HPLC.

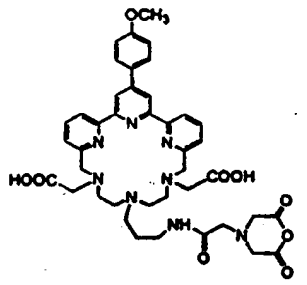
Example 12 Preparation of Escherichia Coli Heat-Stable Enterotoxin (STa) conjugate with 3,9-bis(carboxymethyl)-18-(4-methoxy-phenyl)-6-(3-aminopropyl)-3,6,9,25,26,27-hexaazatetracyclo[19.3.1.1^{11,15}.1^{16,20}]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene γ-t-butoxyglutamic acid amide, (Sta-chelate$_{67}$).

In a 10 ml Reacti-Vial silanized as in Example 4, a solution of 1 part of Escherichia Coli heat-stable enterotoxin (STa) and 1 part of the compound prepared in Example 11 in 10 parts of DMF is treated with one part of 1,1'-carbonyldiimidazole. The symmetrical products of the reaction are separated by preparative HPLC from the desired unsymmetrical conjugate which is treated with trifluoroacetic acid to remove the t-butyl groups. The desired product, STa-chelate$_{67}$, can be isolated by preparative HPLC.

Example 13. N-(3-Methylimidazolium)-carbonylmethyliminodiacetic anhydride trifluoromethane sulfonate.

To a stirred solution of 1,1'-carbonyldiimidazole (1 part) in anydrous nitromethane (2 parts) under nitrogen and cooled to 0°C is added, dropwise, methyl trifluoromethanesulfonate (2 parts). After 20 minutes at 0°C, the reaction mixture is added to a solution of nitrilotriacetic anhydride (1 part; CAS Registry number 33658-49-0) in anhydrous dimethylformamide (3 parts). The reaction mixture is stirred at room temperature under nitrogen for one hour to provide a solution of the desired N-(3-methylimidazolium)carbonylmethyliminodiacetic anhydride trifluoromethane sulfonate which can be used directly.

Example 14. 3,9-bis(carboxymethyl)-18-(4-methoxyphenyl)-6-[3-(4-(2,6-dioxo)morpholino)-methylcarbonylaminopropyl]-3,6,9,25,26,27-hexaazatetracyclo-[19.3.1.1^{11,15}.1^{16,20}]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene.



To a stirred solution of one part of the compound prepared in Example 13 in DMF and nitromethane is added one part of the compound prepared in Example 9. The reaction mixture is stirred under nitrogen at 0°C for 90 minutes and

is then allowed to warm to room temperature and stirred for an additional 90 minutes. One milliliter of water and excess trifluoroacetic acid is added, and the reaction mixture is stirred for one half hour at room temperature. The solvent is then removed under high vacuum, and the residual solid is triturated with water and isolated by filtration. Toluene (10 mL) is added and the crude product is dried by azeotropic distillation to remove water. The crude reaction product is isolated and purified by chromatography on silica gel using step gradients comprising increasing amounts of methanol in methylene chloride and subsequent elution with ammonium hydroxide in methanolic methylene chloride. Fractions containing the desired compound are combined and the solvent is evaporated. The residue is taken up in anhydrous DMF and treated with acetic anhydride. The solvents are removed under high vacuum, and the residue is triturated with cold anhydrous ether and filtered.

Example 15. Preparation of Escherichia Coli Heat-Stable Enterotoxin (STa) conjugate with 3,9-bis(carboxymethyl)-18-(4-methoxyphenyl)-6-[3-(4-(2,6-dioxo)-morpholino)methylcarbonyl-aminopropyl]-3,6,9,25,26,27-hexaazatetracyclo-[19.3.1.$1^{11,15}$.$1^{16,20}$]-heptacosa-11,13,15^{27},16^{26},17,19,21^{25},22,24-nonaene, (STa-chelate$_{70}$).

In a 10 ml Reacti-Vial silanized as in Example 4, a solution of 1 part of Escherichia Coli heat-stable enterotoxin (STa) and 1 part of the compound prepared in Example 14 in 50 parts of anhydrous DMF is heated at 50°C. The reaction is followed by HPLC and the product is isolated by preparative HPLC.

Example 16. Radiolabeling of STa-chelate$_{58}$ with ^{67}Cu.

The method of Example 5 is repeated using STa-chelate$_{58}$ prepared in Example 4 and $^{67}CuCl_2$ in 0. 04 M hydrochloric acid (University of Missouri) in place of $^{90}YCl_3$.

Example 17. Labeling of the conjugate of STa-chelate $_{70}$ with gadolinium ion.

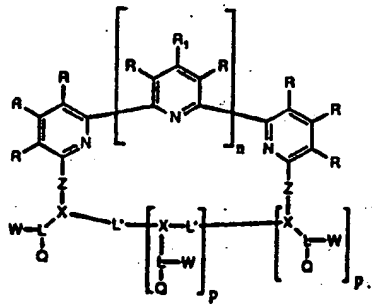
The method of Example 55 of US Patent No. 5760191 is repeated using the STa-chelate$_{70}$ prepared in Example 15 and $GdCl_3$ in place of $EuCl_3$. Progress of the rection is followed spectroscopically.

Example 18. Labelling of STa-chelate$_{70}$ with dysprosium ion.

The method of Example 55 of US Patent No. 5760191 is repeated using the STa-chelate$_{70}$ prepared in Example 15 and $DyCl_3$, in place of $EuCl_3$. Progress of the rection is followed spectroscopically.

Claims

1. A targeting immunoreagent comprising a metal ion, a residue of a complexing agent and a immunoreactive group linked to said complexing agent having the structure.



wherein

each R and R_1 is independently selected from hydrogen, alkyl, alkoxy, hydroxyalkyl, alkoxyalkyl, hydroxyalkyloxy, alkoxyalkyloxy, alkylthio, alkylthioalkyl, alkylthioalkyloxy, hydroxyalkylthio, hydroxyalkylthioalkyl, hydroxyalkylthioalkyloxy, N,N--dialkylamino, N-(hydroxyalkyl)-N-alkylamino, N,N--bis(hydroxyalkyl)amino, N,N-dialkylaminoalkyl, N--(hydroxyalkyl)-N-alkylaminoalkyl, N,N-bis(hydroxyalkyloaminoalkyl, alkylformamido, formamidoalkyl, aryl, alkylaryl, alkoxyaryl, hydroxyalkylaryl, alkoxyalkylaryl, hydroxyalkyloxyaryl, alkoxyalkyloxyaryl, alkylthioaryl, hydroxyalkylthioaryl, hydroxyalkylthioalkylaryl, hydroxyalkylthioalkyloxyaryl, aralkyl, aralkyloxy, alkoxyaralkyl, alkoxyaralkyloxy, aryloxy, alkylarylaxy, alkoxyaryloxy, and heterocyclyl;

each Q is independently selected from hydrogen, alkyl, hydroxyl, carboxyl, carboxyalkyl, hydroxyalkyl, alkylthioalkyl, sulfhydryl, thioalkyl, alkoxy, alkylthio, alkylamino, aminoalkyl, aminoalkylaminoalkyl, hydroxy-alkylaminoalkyl, hydroxylaminoalkyl, hydroxamido, formamidoalkyl, alkylformamido, aryl,

including substituted aryl, aryloxy, heterocyclyl, carbonyliminodiacetic acid, methyle eiminodiacetic acid, methylenethioethylene-iminodiacetic acid, carboxyalkylthioalkyl, a residue of ethylenediaminetetraacetic acid (EDTA), a residue of diethylenetriaminepentaacetic acid (DTPA), hydrazinylidenediacetic acid, and a salt of any of the foregoing acids;

each Z is independently selected from a heteroatom with a valence of two, a heteroatom with a valence of three, an alkylene group, an alkylene group bonded to a heteroatom having a valence of two, and an alkylene group bonded to a heteroatom having a valence of three;

each X is independently selected from nitrogen and a residue of an alkylene group;

each W is independently selected from hydrogen and a substituent that comprises a protein reactive group;

each L' is independently selected from a chemical bond and an intra-ring linking group;

each L is independently selected from a residue of an alkylene group and an extra-ring inking group;

n is 1, 2, 3 or 4; and

each p is independently 0, 1, 2, 3 or 4;

provided that only one W is a protein reactive group; the L bonded to the W that is a protein reactive group contains 1, 2, or 3 carbon atoms and connects X to a heteroatom capable of participating in the chelation of a metal ion; and when X is a nitrogen and a heteroatom of Z is bonded to X, the heteroatom of Z is also nitrogen;

and the immunoreactive group is an ST receptor binding moiety.

2. The immunoreagent of claim 1 wherein said immunoreactive group is linked through a linking group to said complexing agent wherein the linking group between the complexing agent and the immunoreactive group comprises the residue of the protein reactive group on the complexing agent.

3. The immunoreagent of claim 1 or 2 wherein n=1.

4. The immunoreagent of any one of claims 1 to 3 wherein the

protein reactive group is selected from the group consisting of amino; aminoalkyl; aminoaryl; alkylamino; arylamino; hydrazino; alkylhydrazino; arylhydrazino; carbazido; semicarbazido; thiocarbazido; hydrazidoalkyloxy; azidocarbonylalkyloxy; aryloxycarbonyloxyalkyloxy; triazines; aryloxycarbonyl(polyoxyalkyl)oxy; thiosemicarbazido; sulfhydryl; sulfhydrylalkyl; sulfhydrylaryl; hydroxy; carboxy; carboxyalkyl; carboxyaryl; active halogen containing groups; 2-leaving group-substituted ethylsulfonyl and ethylcarbonyl; vinylsulfonyl; vinyl sulfonylalkyloxy; vinyl sulfonylalkylpoly(oxyalkyl)oxy; vinylcarbonyl; oxiranyl; isocyanato; isothiocyanato; aldehydo; aziridinyl; succinimidoxycarbonyl; activated acyl groups; anhydride groups; thioester groups; active carbonates; sulfonic acid esters; phosphoramidates; cyanuric monochlorides and dichlorides; and groups that can be linked to protein or modified protein by use of a crosslinking agent.

5. The immunoreagent of claim 4 wherein the protein reactive group is selected from the group consisting of chloromethylphenyl, chloromethylcarbonyl, iodomethylcarbonyl, 2-chloroethylsulfonyl, 2-chloroethylcarbonyl, carboxylic acid halide groups, alkylhydrazino, arylhydrazino, semicarbazido, thiocarbazido, thiosemicarbazido, isocyanato and isothiocyanato, vinyl sulfonylalkyloxy, vinyl sulfonylalkyl(polyoxyalkyl)oxy, amidatoalkyloxy, hydrazidoalkyloxy, azidocarbonylalkyloxy, aryloxycarbonyloxyalkyloxy, aryloxycarbonyl(poly-oxyalkyl)oxy, 4,6-dichloro-2-triazinyloxy, dichlorotriazinyl-(polyoxyalkyl)oxy, 4-alkoxy-6-chloro-2-triazinyloxy, 4-alkoxy-6-chloro-2-triazinyl(polyoxyalkyl)oxy, formylalkyl, aminoalkyl, thioalkyimidoaminoalkyloxy, active esters, active anhydrides, nitrophenylcarbonates, arylcarbonatoaryl, alkylcarbonatoaryl, arylcarbonatoalkyl, alkylcarbonatoalkyl, mixed anhydrides, thioalkylcarbonylaminoalkyloxy, succinimidoxycarbonyl, maleimidoalkylcarbonylaminoalkyloxy, azido, 4,6-dichloro-2-triazinylamino, 4,6-dichloro-2-triazinyloxyalkyl, 4,6-dichloro-2-triazinyloxyaryl, 4,6-dichlorotriazinyl-2-oxy(polyalkylaxy), iodoalkylcarbonylamino, alkylamino, arylamino, amidatoalkylamino, and amidatoarylalkylamino.

6 The immunoreagent of claim 5 wherein the protein reactive group is selected from the group consisting of sulfhydryl, amino, aryl carbonato alkyl, active esters, isothiocyanato and thiosemicarbazido.

7. The immunoreagent of any one of claims 1 to 6 wherein said metal ion is a radionuclide ion.

8. The immunoreagent of claim 7 wherein the radionuclide ion is selected from the group consisting of Sc, Fe, Pb, Ga, Y, Bi, Lu, Mn, Cu, Cr, Zn, Ge, Mo, Tc, Ru In, Sn, Sm, Sr, Eu, Dy, Sb, W, Re, Po, Ta and Tl ions.

9. The immunoreagent of claim 8 wherein the radionuclide ion is selected from the group consisting of ^{44}Sc, ^{111}In, ^{212}Pb, ^{68}Ga, ^{90}Y, ^{177}Lu, ^{186}Re, ^{188}Re, ^{64}Cu, ^{67}Cu, ^{99m}Tc, ^{87}Y and ^{212}Bi ions.

10. The immunoreagent of claim 9 wherein the metal radionuclide ion is 90Y+++.

11. The immunoreagent of any one of claims 1 to 6 wherein the metal ion is a paramagnetic metal ion.

12. The immunoreagent of claim 11 wherein the paramagnetic metal ion is selected from Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu

13. The immunoreagent of claim 12 wherein the paramagnetic metal ion is selected from Cr^{+3}, Cr^{+2}, V^{+2}, Mn^{+3}, Mn^{+2}, Fe^{+3}, Fe^{+2}, Co^{+2}, Gd^{+3}, and Dy^{+3}

14. The immunoreagent of any one of claims 1 to 6 wherein the metal ion is a fluorescent metal ion.

15. The immunoreagent of claim 14 wherein the fluorescent metal ion is selected from

16. The immunoreagent of claim 15 wherein the fluorescent metal ion is Eu^{3+}.

17. A diagnostic imaging or therapeutic composition comprising a immunoreagent of any one of claims 1 to 16 and a pharmaceutically carrier therefor.

18. A method for diagnostic imaging a site in a patient comprising the steps of

a) administering to the patient an effective amount of a radioactive immunoreagent of any one of claims 7 to 10 capable of targeting said site and

b) imagewise activating a radiation sensitive element or device with the radiation emitted from the targeted site.

19. A method for treating disease sites in a patient comprising the steps of administering to the patient or a specimen from the patient an effective amount of a therapeutic composition of claim 17.

20. A method for diagnostic imaging a site in a patient comprising the steps of

a) administering to the patient an effective amount of a targeting paramagnetic immunoreagent of any one of claims 11 to 13 capable of targeting said site and

b) imagewise activating a nuclear magnetic resonance detection sensor element or device which is sensitive to a change in one or more nuclear magnetic relaxation properties of an isotope at said site while exposed to a controlled magnetic field environment.

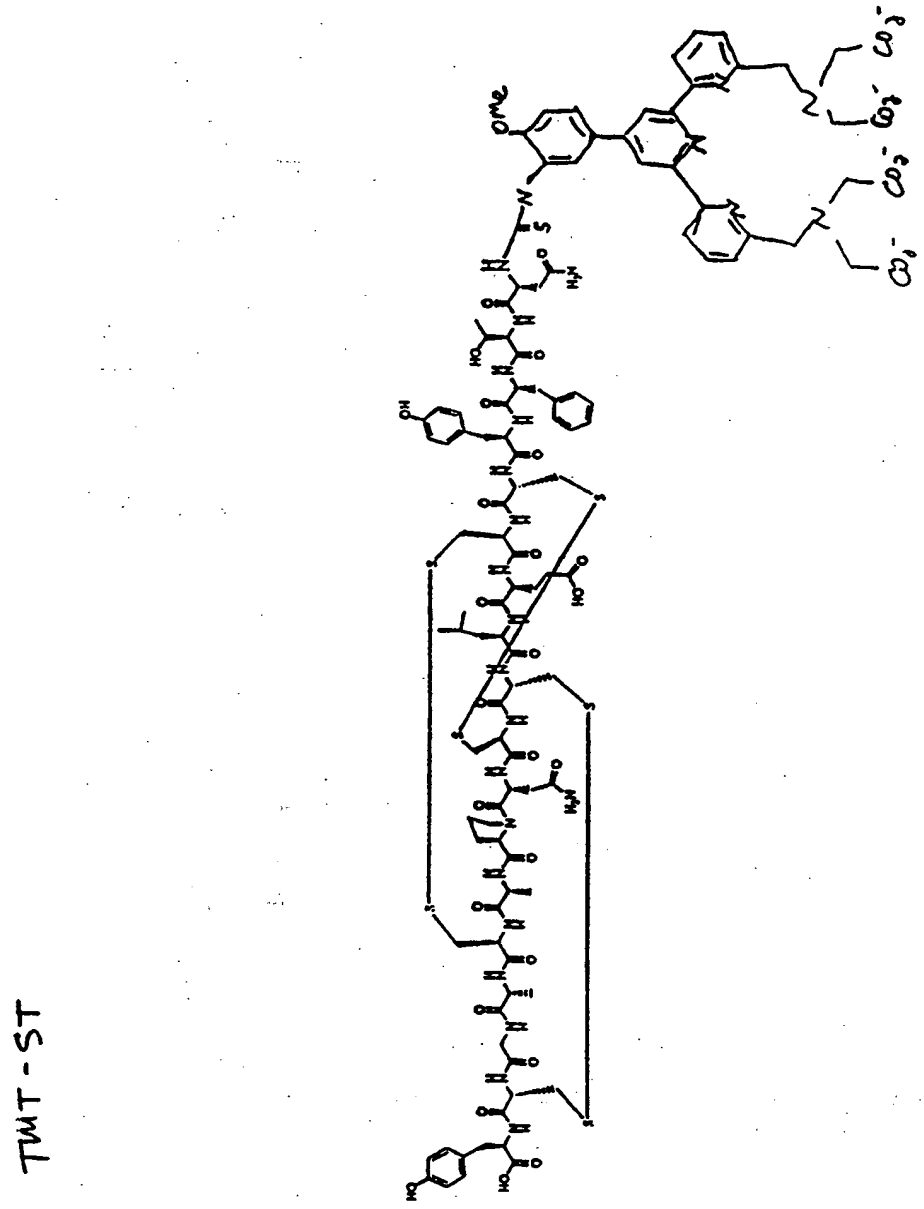


FIGURE 1

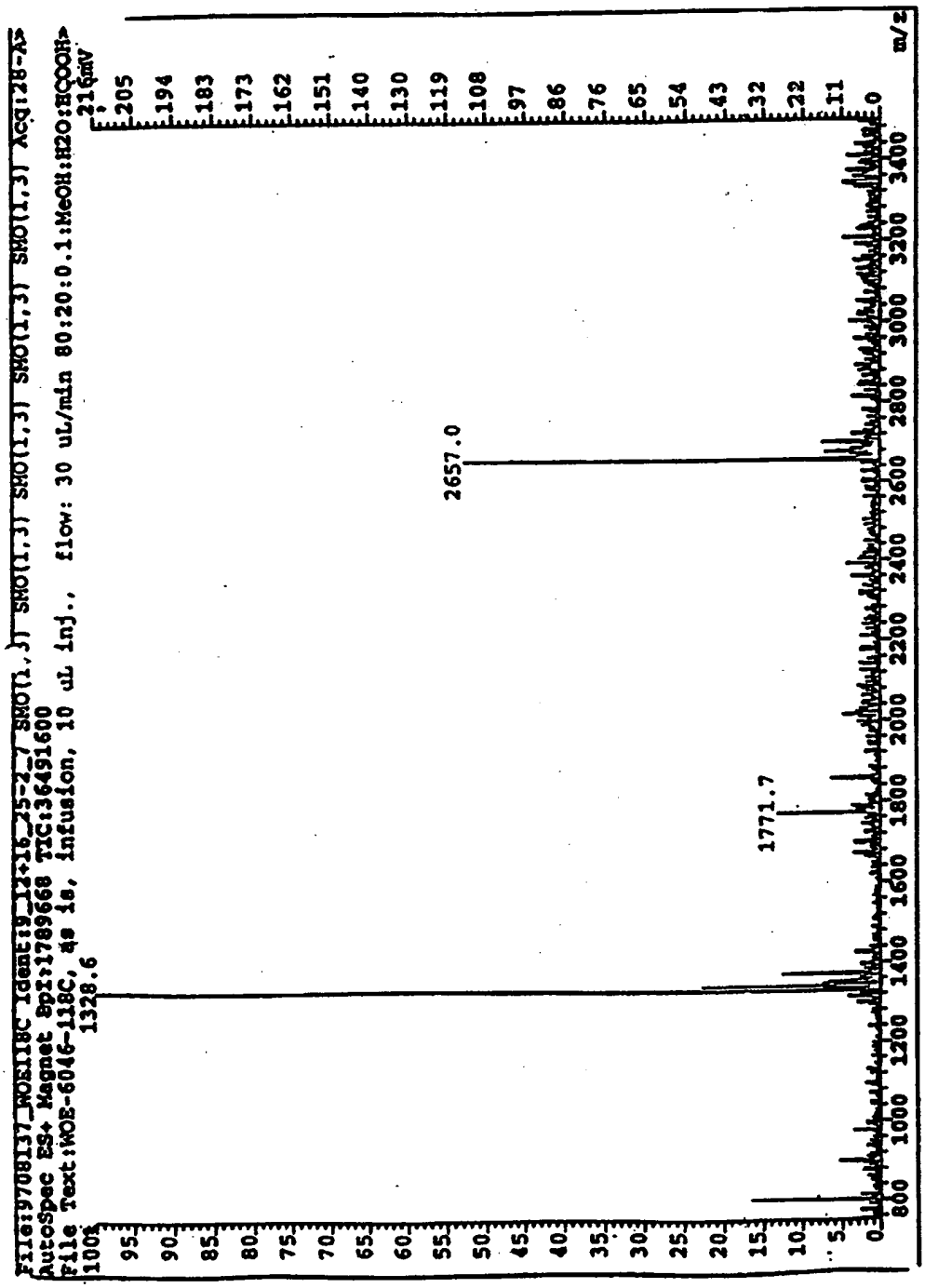

File:970813?_ROE118C_Ident19_[1+]16_25-2_7 SMOT(1,3) SMOT(1,3) SMOT(1,3) SMOT(1,3) SMOT(1,3) Acq:28-A
AutoSpec ES+ Magnet Bpi:1789668 TIC:36491600
File Text:ROE-6046-118C, 4s is, infusion, 10 uL inj., flow: 30 uL/min 80:20:0.1:MeOH:H2O:HCOOH
216mV
1328.6
2657.0
1771.7
m/z

FIGURE 2

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 99/00396

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K51/10 A61K49/00 A61K51/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 08494 A (STERLING WINTHROP INC) 29 May 1992 (1992-05-29) claims	1
E	WO 99 21587 A (MATTHEWS DEREK PETER ;NYCOMED IMAGING AS (NO); DELECKI DANIEL JOSE) 6 May 1999 (1999-05-06) claims	1-20
X	WO 95 11694 A (UNIV JEFFERSON ;WALDMAN SCOTT A (US)) 4 May 1995 (1995-05-04) claims	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 1999	21/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Berte, M

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/ 00396

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
See FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant. this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/00396

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